INCORPORATION OF NEW TECHNIQUES IN ANIMAL BREEDING PROGRAMMES WITH AN EMPHASIS ON DAIRY CATTLE

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DECLARATION

I declare that this thesis is my own composition and that the research described in it is my own work, except where otherwise stated.

John Francis Kearney, 2006

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ABSTRACT

Animal breeding programmes often face new challenges either from problems created by past selection strategies or the emergence of new technologies. Two such examples are the increase in inbreeding now seen in many livestock populations and the availability of molecular genetic information, which can be used to enhance genetic gain. The general objectives of this thesis were a) to determine the current levels of inbreeding in UK dairy cattle and the value of optimised selection as a method to control inbreeding and b) to evaluate certain aspects in relation to the use of molecular genetic information in animal breeding programmes.

Recently, concerns have been raised over the degree of inbreeding in dairy cattle populations. A study of the inbreeding of UK Holstein cows was undertaken to assess the current levels and trends in inbreeding in this breed. Results showed that the rate of inbreeding has increased considerably since the early nineties. The annual rate from 1992 to 2003) was 0.17% while the rate from 1968 to 1991 was just 0.03%. Optimisation procedures, which have been developed to maximise the rate of genetic gain at a predefined rate of inbreeding, were applied to determine if such procedures would be useful to in a dairy cattle context. The results showed that the procedures were able to generate more genetic gain at current rates of inbreeding or alternatively to reduce the rate of inbreeding at the current rate of genetic gain.

The emergence of new molecular genetic techniques has meant that many resources have been devoted to the mapping of quantitative trait loci (QTL) of economic importance in most domestic livestock species. The detection of such loci provides a new source of information that can be used in conjunction with traditional methods to enhance the selection process and increase genetic gain. In this thesis a method is described to assess the benefits of using DNA tests at the commercial herd level. This method can be used for a variety of situations and two case studies were examined to illustrate the usefulness of the method. The first study dealt with the situation in which a breeder chooses to use a sire that is a carrier for a deleterious allele. The second study dealt with the value of sires that are carriers of a beneficial allele for which a premium is paid. The method would be very useful to predict the costs or benefits associated with using identified loci in commercial herds.

The usefulness of QTL in breeding programs will depend on, among other things, the mode of action of a QTL on specific trait(s). An estimate of the genetic correlation equal to zero between two traits suggests that the traits in question are controlled independently. However, through stochastic simulation, it is shown that a pleiotropic QTL can result in a QTL segregating at intermediate frequencies in a population when the estimated polygenic correlation is zero. As a consequence, it is possible for a deleterious allele to remain in a population for many generations, when it might otherwise be expected to be removed via natural selection. The expected outcomes from using pleiotropic QTL in selection were also assessed. In general, the expected gain is a function of the mode of action of the QTL on traits it affects. It can lead to less gain compared to selection ignoring the QTL where the QTL has a negative effect on the fitness of the animals.

CHAPTER ONE

General Introduction

1.1 Introduction

Genetic improvement of livestock is a constantly evolving area with animal breeders facing challenges, not only as a result of past selection decisions but also with the emergence of the latest technology, and the need to incorporate this to increase genetic gain. This general introduction outlines some of the current problems and developments in livestock genetics. Specifically, two areas are highlighted. The first deals with the increasing concern regarding inbreeding, while the second deals with the emergence of molecular genetic information that can be used in breeding programmes. Chapters two and three look specifically at situations pertinent to dairy cattle, while chapters four and five refer to more general situations.

1.2 Inbreeding

Traditionally, breeding programmes have relied on the collection and evaluation of phenotypic information for livestock improvement. This information can be partitioned, via statistical methodologies such as Best Linear Unbiased Prediction (BLUP), to provide an estimate of the genetic merit (or breeding value) of individuals in a population. Elite animals can then be selected, with very high accuracy, to be parents of the next generation, based on these estimated breeding values (EBV). Such selection programmes have been applied to many livestock species, most notably for cattle, sheep, pigs, and poultry. These programmes have been highly successful and have greatly increased the production levels of these species. For example, in the US and the Netherlands, average milk production per lactation has approximately doubled (phenotypically) in 40-50 years of selection. Genetic progress has been even greater. For example, the average breeding value of a

US Holstein cow for milk production was -6818kg of milk in 1957, but the average breeding value is currently 667kg of milk (USDA, 2006). Improvements of similar or greater magnitude have been reported for growth rate for broilers and pigs (Rauw et al., 1998).

However, one of the consequences of traditional breeding programmes has been the increased rate of inbreeding. Statistical methods such as BLUP usually result in the coselection of relatives. Elite animals are identified with high accuracy, and these animals are preferentially selected for by breeders. Selection for production traits only has exacerbated this problem, as favourable alleles for traits other than those in the current selection objective, may be selected against and lost from the population irreplaceably. The use of modern reproductive and DNA technologies have the potential to identify and replicate more elite animals faster, which will lead to faster rates of inbreeding unless due account of the problem is taken.

1.2.1 Levels of inbreeding

Accurate and intense selection is the primary reason for the large increases in average relationships between individuals and inbreeding in livestock populations. For example, researchers have reported that the average relationship amongst females in US Holstein dairy population has increased from 3.4% in 1928 to 10% in 1990 (Young and Seykora, 1994). Furthermore, the same authors reported that two sires accounted for over one quarter of the genes segregating in the population. Currently, the average inbreeding coefficient of the Holstein population is approximately 5% (AIPL, USDA, 2002). Thompson et al. (2000) reported that the

inbreeding coefficient of the population has increased geometrically over the last ten years. The average annual increase in inbreeding is now 0.2% compared to just 0.04% during the 1970s and 0.12% during the 1980s. The increase in relationships among individuals, and subsequently inbreeding, has resulted from the intense selection of few sires, rather than from a reduction in the total population size (Weigel, 2001). For example, the levels of inbreeding in numerically large breeds like the Holstein population are similar to that of smaller breeds such as Guernsey and Ayrshire. Widespread international trade in semen for the most popular Holstein bulls means that the genetic base is further reduced. Some bulls have sired over 250,000 daughters and 3,000 progeny test sons worldwide (Weigel, 2001).

1.2.2 Consequences of inbreeding

Increasing rates of inbreeding is a concern for a number of reasons. Firstly, as the degree of inbreeding increases animal performance can be reduced. This phenomenon, known as inbreeding depression, has been well documented in livestock populations for various traits. The genetic basis of inbreeding depression is not yet fully understood and two potential hypothesis have been proposed. The dominance hypothesis inplies that inbreeding depression is caused by the expression of deleterious recessive genes in homoygous individuals while the overdominance hypothesis implies that the individuals in the heterozygous state have increase fitness relative to the homozygotes. Either way it seems that inbreeding depression is a consequence of dominance, and is expected to effect traits that exhibit more dominance variation, especially fitness related traits.

Inbreeding depression has been shown too occur for traits of livestock. For example, inbreeding has been shown to decrease milk production by approximately 9-26 kg of milk per lactation for each 1% increase in inbreeding (Thompson et al. 2000, Smith et al., 1998, Miglor et al., 1994). Smith et al. (1998) reported an economic loss in relative net income of $12.40 per 1% increase in inbreeding over the lifetime of a cow. Thompson et al. (2000) found that the greatest negative effect of inbreeding occurred early in the life of the animal and early in lactation. Wall et al. (2005) found a significant effect of inbreeding on fertility and correlated traits in Holsteins, and the effect of inbreeding was more severe at higher levels of inbreeding.

Secondly, the increase in homozygosity due to inbreeding has also led to an increase in genetic disorders observed in livestock populations. Classical examples in dairy cattle are complex vertebral malformation (CVM), bovine leukocyte adhesion deficiency (BLAD), and deficiency of uridine monophosphate synthase (DUMPS) (Nicholas, 1996). The origin of both CVM and BLAD can be traced to a single sire that was extensively used as a sire of sons. These genetic disorders are financially very expensive due to losses in production, mortality, and in certain cases, veterinary costs.

Thirdly, inbreeding reduces the genetic variability in populations. Genetic variability is essential in order to achieve response to selection. Increasing inbreeding will ultimately lead to reduced variation thereby limiting response to selection in the long-term. High rates of inbreeding will deplete genetic variation fast; therefore methods need to be applied for balancing variation lost by inbreeding and that gained by mutation. Operational tools to aid the design of a breeding programme to

maximise genetic gain while imposing specific restrictions on the rate of inbreeding are available and are described in more detail below.

1.2.3 Control of inbreeding

Several methods for controlling inbreeding employing modifications to selection or mating decisions have been proposed. For any strategy aimed at controlling the rate of inbreeding it is important that a desirable level of genetic gain is maintained. Breeding companies may see inbreeding as a long term problem, whereas their business survival is based on short term profit through maximising genetic gain. However, in the future, their customers may demand stock that does not lead to high levels of inbreeding in the offspring, therefore they need to be mindful of this to secure their future success.

1.2.4 Modifications to mating strategies

Woolliams (1989) proposed the use of factorial mating designs where dams are mated to more than one sire and sires are mated to more than one dam. The factorial design implies a reduction of the number of full-sib progeny and an increase in the number of half-sibs in comparison with a hierarchical design (Sorensen et al., 2005) and leads to a reduction in inbreeding with little or no reduction in genetic response. Caballero et al. (1996) used simulations to compare two systems of mating, compensatory and minimum coancestry, for their ability to reduce inbreeding in selected populations. The former system involves mating between individuals from the largest selected families to individuals from the smallest, while the latter involves matings that minimise the average pairwise coancestry of selected individuals. Both

mating systems were successful in reducing the rates of inbreeding with little or no reduction in response. The effectiveness of each system differed according to the selection strategy (phenotypic or BLUP), the population size and structure, and the heritability of the trait.

1.2.5 Modification to selection

Selection strategies offer higher potential to control inbreeding than mating strategies. Some authors have proposed certain modifications to the BLUP procedure. The use of an upwardly biased estimate of heritability has been shown to be a simple, yet efficient method of reducing rates of inbreeding with little effect on rates of gain (Toro and Perez-Enciso, 1990; Grundy et al. 1994). Inflating the estimate of heritability increases the weight on an individuals own performance and decreases the weight on familial information, thereby reducing the chances of co-selecting relatives. This is especially true for traits with low heritabilty. Villanueva et al. (1994) successfully reduced rates of inbreeding, with little or no loss in genetic gain by selecting on a modified index that reduced the weight given to family information, specifically the weight given to parental EBV.

The methods described above achieve desired results, however, in practice, it may be difficult to determine optimal parameters for such modifications. For example, what value should the biased heritability be set to and what amount of family information should be subtracted from an individual's EBV? During the last decade a method has been developed to optimise the contributions of selected parents to the next generation while restricting the rates of inbreeding (Meuwisswen, 1997, Grundy et

al. 1998). With this method animals are selected such that the optimum mating proportions for each candidate can be obtained to maximise gain for a given rate of inbreeding. The method uses the best estimates of breeding values (i.e. BLUP EBV) of the selection candidates and takes into account all genetic relationships. Simulation studies have shown that at the same level of inbreeding the genetic gains are 21 to 60% greater for the optimised selection than for selection exclusively based on BLUP EBV (Meuwissen, 1997). This method has also been shown to work for overlapping generations (Grundy et al., 2000, Sonesson and Meuwissen, 2000). Breeding companies are generally interested in maximising short term genetic gain while not paying much attention to accumulating inbreeding and loss of genetic variation. However, breeding programmes all impose some restrictions on inbreeding but this can lead to suboptimal gains. Optimised selection is still very useful in situations where inbreeding is not of concern as it produces the maximum possible gains at the current inbreeding rates.

1.3 The Role of Molecular Genetics in Animal Breeding

While I have discussed some of the challenges posed to the traditional breeding programmes, a new era for animal breeding is fast approaching. Over the last decade or so advanced DNA technologies (in particular DNA markers) are becoming available to the livestock breeding industries. The rapid advances in molecular genetic technologies have greatly increased the chances of identifying quantitative trait loci (QTL) or of markers linked to such loci in livestock species. There are many examples of QTL identified in livestock populations (e.g. Rothschild et al., 1996; Grobet et al., 1997; Ashwell and Van Tassell, 1999; Casas et al. 2001; Cassady

et al. 2001; Ikonen et al. 2001; Dekkers, 2004). Grisart et al. (2002) reported on the positional cloning of a QTL (DGAT1) with a major effect on milk yield and composition. This result was significant since some scientists theorised that it was unlikely that QTL of large effects for highly selected traits such as milk production, were still segregating in the population. However, it was shown by Grisart et al. (2002) that the DGAT1 polymorphism was neutral with respect to the selection indices in both the Netherlands and New Zealand hence why the alleles were segregating at intermediate frequencies in these populations.

There are a few ways in which breeders could ultimately utilise molecular genetic markers (Dekkers and Hospital, 2002). Firstly, markers can be used to assist in the introduction of a single gene with a favourable effect to one population from another (marker-assisted introgression or MAI). Gene introgression has been proposed to introduce high-litter-size alleles from the Chinese Meishan pig breed into high performance commercial breeds (Rothschild et al., 1994) and disease-resistance alleles from tropically adapted breeds into high performance but susceptible breeds of beef cattle (Frisch, 1994).

Secondly, markers could be used to accelerate selection for a particular trait within a population (marker-assisted selection or MAS). Fernando and Grossman (1989) proposed a method for utilising genetic marker information in BLUP genetic evaluations. With this method, the EBV of an individual is calculated as the sum of its breeding value due to the polygenes and the breeding value due to the QTL. This method has been the basis of several simulation studies carried out to evaluate MAS for various scenarios. These studies found extra (although variable) gains,

particularly for sex limited and lowly heritable traits (e.g. Kashi et al., 1990; Meuwissen and van Arendonk, 1992; Ruane and Colleau, 1995; Meuwissen and Goddard, 1996), for MAS over conventional selection. In general, the magnitude of the improvement in the response to selection is a function of the number of generations of selection, the population size and structure, the heritability of the trait, the number of markers, and the magnitude of the effect of the QTL linked to the markers (Davis and DeNise, 1998). However, inclusion the markers in conventional BLUP evaluations have some limitations, most notably the availability of genotype information on only a small proportion of animals in a national genetic evaluation programme. Also, the choice of which animals to genotype has to be considered. It is plausible that cost effective high-throughput genotyping will be available in the future to overcome some of these limitations. Another limitation would be the extensive changes that would be required for existing genetic evaluation procedures, component estimation, and computer resources (Dekkers, 2004)

One of the first proposed benefits of MAS was the use of markers for the pre-selection of young unproven full-sib dairy bulls. Usually, the choice between full-sib young bulls is at random as their initial EBV would be the same based on parental information. However, with the use of DNA markers these bulls can be genotyped for specific markers relating to for example, increased milk production. The costs associated with progeny testing could be reduced substantially, by only selecting the most promising bulls that carry the markers of interest. Mackinnon and Georges (1998) and Kaski et al. (1990) have shown additional responses of 8-30% over that obtained from progeny testing alone, primarily through increased selection intensity and reduced generation intervals.

1.3.1 Economic assessment of MAS

As with any new technology, its implementation will depend to a certain extent on the cost of the technology and the benefit attainable from its implementation. While vast amounts of financial resources are currently directed to the search of QTL markers linked to traits of interest, implementation of MAS will require the genotyping of many animals. If MAS is to be successful, increased returns in terms of genetic gain from using this technology must outweigh the cost of genotyping. A few studies have looked at the economic implications of MAS in nucleus breeding schemes. For example, Amer and Villanueva (2000) assessed the economic benefit of using a major biallelic gene in a nucleus breeding programme, including a constraint on the rate of inbreeding. Benefits to the breeding company were measured in terms of Net Present Value (NPV) that accounted for the planning horizon, discount rate, fixed costs, time lag, sale price, and genotyping costs. NPV was calculated for the sale of breeding males carrying one or two copies of the favourable allele. They found that the NPV was greater than the corresponding schemes ignoring the major gene. Hayes and Goddard (2003) found that MAS was slightly more profitable than non-MAS (where profitability was the difference in response from MAS and non-MAS minus the costs of genotyping) in a pig breeding scheme that included a QTL that affected four independent traits.

Currently, DNA markers are commercially available for traits of importance in livestock populations (Dekkers, 2004) and are being marketed at the farm level. Some of these markers include the leptin gene, DGAT1 for milk yield and bovine growth hormone receptor (Igenity, 2006). However, the benefits of using these markers at the farm level have yet to be determined.

1.3.2 Pleiotropic QTL

The use of marker information in breeding programs has focussed primarily on single trait situations (e.g. Meuwissen and van Arendonk, 1992; Ruane and Colleau, 1996; Villanueva et al, 1999 and 2004). However, in most domestic livestock breeding programmes the breeding objective includes several traits that may be affected by the same QTL. For example, one of the main causes for the existence of a genetic correlation between traits is pleiotropy (Falconer and MacKay, 1996). Therefore in the context of MAS it is necessary to identify pleiotropic QTL to avoid negative effects of selection on important traits. Methods to identify pleiotropic QTL have been reported (e.g. Stearns et al., 2005; Xu et al, 2005; Schrooten et al., 2002; Knott and Haley, 2000), and these advances could be critical to the success of MAS in the future. Amongst others, examples of pleiotropic QTL have been reported in dairy cattle (Schrooten et al., 2004, Grisart et al., 2002), in pigs (Stearns et al., 2005), and in chickens (Navarro et al., 2006). Grisart et al., (2002) found a gene (DGAT1) that was repsonible for increased fat yield, while decreasing milk and protein yield, despite the overall positive correlation between the three traits. In the study of Navarro et al. (2006) it appeared that a pleiotropic QTL affecting production and an indicator for ascites resistance (blood oxygen saturation) was segregating in the population. They also concluded that the mode of action of the QTL was overdominant for production and dominant for the ascites indicator. This poses some interesting questions in relation to the expectation of changes in QTL allelic frequencies and in genetic means for traits affected by the QTL when using such QTL in selection.

To date, few studies have looked at the implications of using pleiotropic QTL in breeding programmes. De Koning and Weller (1994) simulated a QTL that had an effect on either a single trait or on two traits. Genetic gain was higher when the QTL had an effect on both taits and was highest when the genetic correlation between the traits was most negative. About 10% more response was achieved when the QTL had an effect on both traits when the genetic and phenotypic correlations between the traits was zero. Verrier (2001) investigated MAS for a pleiotropic QTL that affected two negatively correlated traits (with a genetic correlation of -0.4) and where no males had records for one of the traits. He found that, when compared with selection ignoring the QTL, MAS gave higher response in the initial generations but lower response after five generations. Both studies looked at pleiotropic QTL that acted additively on both traits under selection. However, very different result might be expected for the model of Navarro et al. (2006).

1.4 Summary and objectives

This general introduction has focused on some of the aspects that are important in animal breeding in many livestock species. Inbreeding and the use of molecular genetic information are two areas that have received particular attention recently. Inbreeding is, in some way, the manifestation of successful selection, while molecular genetics has the potential to provide vast amounts of information on the underlying mechanisms that has driven selection to date.

Inbreeding is playing an increasingly important role in livestock species. Losses through inbreeding depression and genetic defects can be substantial and the trend in inbreeding needs to be slowed or reversed. Recent experiences in dairy cattle have shown how even in numerically large breeds, increases in the rate of inbreeding may become a problem. The effective population in the Holstein population is currently only about 50 (Brotherstone and Goddard, 2005). In chapter two, the average inbreeding coefficient and rate of inbreeding of the UK Holstein population are estimated and the benefits of applying optimised selection to select progeny test bulls for maximising genetic progress while restricting the rate of inbreeding are investigated.

Many studies are being conducted to map QTL for economically important traits and the incorporation of molecular information with traditional selection schemes appears to the appropriate step forward for animal breeders. Already, the use of single gene tests has allowed potentially harmful recessive diseases such as BLAD, and CVM to be identified early in an animal's life. DNA markers for several other traits (e.g. feed intake, milk production, meat quality) are now available for commerical use on farms (e.g. Igenity, 2006). However, little work has been carried out the economic benefit of using these markers at the herd level. In chapter three, a method to assess the benefits of using identified loci in dairy herds is proposed and two examples of using the identified loci are used to illustrate the usefulness of the method.

The benefits of MAS over conventional selection have mainly been assesed via simulation. In general, extra genetic gain can be achieved when using QTL

information in breeding schemes both in the short-term and in the longer term (Villanueva et al., 2004). Most of these studies have looked at QTL that affect a single trait. However, more sophisticated techniques are being developed at both the molecular and statisical level to determine if the QTL affects more than one trait. Results from MAS could be different if a single QTL is assumed, when in fact it is a pleiotropic QTL. It could result in unexpected changes in the allele frequency and the genetic gain in the trait under selection. Chapter four investigates the effect of artificial selection for production on the frequency of a QTL that has a pleiotropic effect on production and disease susceptibility. In chapter five, the genetic gain attainable from selection schemes when using information on the pleiotropic QTL with different modes of action on both traits is compared to schemes ignoring the QTL.

CHAPTER TWO

INBREEDING TRENDS AND APPLICATION OF OPTIMISED SELECTION IN THE UK HOLSTEIN POPULATION

2.1 Introduction

Despite being a numerically large breed, inbreeding of Holstein populations is increasing and becoming a concern in many countries. In the US, the current inbreeding coefficient of Holstein cows is 5% (AIPL, 2003), representing a doubling since 1990. In Canada, the current average inbreeding is also around 5%. From 1990 to 2000 the rate of increase in inbreeding was 0.25%/yr, a five-fold increase since the previous decade (CDN, 2003). Estimates of average inbreeding and rates of inbreeding for the UK dairy population are not routinely published. Roughsedge et al. (1999) reported an average inbreeding coefficient of 0.43% for the British Holstein – Friesian population for animals born in 1997. However, these results were based on multiple random samples of 2000 cows, but more importantly there was a lack in the number of generations of complete pedigree of foreign sires.

The increase in inbreeding in the Holstein population can be attributed to a number of factors including i) the tendency to co-select related animals as a result of using BLUP estimated breeding values, ii) the use of fewer sires and dams facilitated by AI and multiple ovulation and embryo transfer, and iii) selection for only a few traits such as milk yield and type which are usually positively correlated (e.g., Misztal et al., 1992; Klassen et al., 1992; Visscher and Goddard, 1995). For example, Young and Seykora (1996) identified two sires that together accounted for nearly one-quarter of the genes of registered US Holstein animals born in 1990. They are still the two most highly related sires to Holstein cows in the US (AIPL, 2003), and were recently reported as appearing in more than 95% of all pedigrees of inbred German Holstein cows (Swalve et al., 2003).

The consequences of inbreeding are manifested in terms of inbreeding depression, an increase in undesirable recessive disorders and a loss in genetic variation. Numerous studies have shown a decrease in performance for production (e.g., Thompson et al., 2000; Smith et al., 1998) and non-production traits (e.g., Wall et al. 2005; Cassell et al., 2003) with increasing inbreeding. Similarly, the prevalence of known genetic disorders such as Complex Vertebral Malformation (CVM) and Bovine Leucocyte Adhesion Deficiency (BLAD) and the appearance of other new disorders may increase as inbreeding becomes more widespread. These consequences have the potential to be expensive in terms of production losses due to inbreeding depression, and veterinary and culling costs associated with genetic disorders. Also, the loss of genetic variation as a result of inbreeding is important as it limits the ability to improve traits through genetic selection.

Several strategies to control the rate at which inbreeding accumulates have been proposed in the past (see Weigel, 2001; and Villanueva et al. 2004 for reviews). They include modifications of mating schemes (e.g., factorial mating designs) and modifications to selection methods (e.g., using BLUP with an artificially inflated estimate of heritability). However, the most efficient approach is to manage simultaneously genetic gain and inbreeding in selection decisions and optimise contributions of candidates for maximising genetic gain while restricting at the same time the rate of inbreeding. The potential application of optimised contributions in real livestock populations has been investigated in several species. Weigel and Lin (2002), concluded that optimised selection could be used to control the rate of

inbreeding in the US dairy cattle populations, but did not compare the potential genetic gain achieved by optimised selection to current genetic gain at the current inbreeding rate. In a study of a beef and sheep population, Avendaño et al., (2003) found that optimised selection can lead to increased genetic gains of 17% (beef cattle) and 30% (sheep) when compared to conventional BLUP truncation selection at the same rate of inbreeding.

The objective of this study was to determine the current level and rates of inbreeding and to assess the potential of using optimised selection procedures in the UK dairy population. It is expected that the bull breeding path of selection will control the long-term genetic response and rates of inbreeding in the whole population (Goddard and Smith, 1990; Weigel and Lin, 2002), therefore we concentrate on using optimised selection to manage the relationships among young bulls that would be entering progeny testing schemes.

2.2 Material and Methods

2.2.1 Inbreeding

Pedigrees were extracted from the Holstein UK database for animals born since 1940. The database includes all registered pedigee and grading up animals, and all imported foreign males and females with progeny in the UK. Percentage Holstein was also available for each animal. Inbreeding coefficients for each animal were calculated using the algorithm of Meuwissen and Luo (1992) for 330,037 males and 7,029,545 females born from 1940 to 2002. Mean inbreeding was calculated per year based on the year of birth of the animals. In order to assess the quality of the data to

estimate inbreeding a measure of pedigree completeness was calculated for each animal according to MacCluer et al. (1983). In 2002, 96% of animals had three or more generations of complete pedigree information and 85% had four or more generations of complete information. The rate of inbreeding was calculated by regressing the mean inbreeding on the year of birth for three time periods: i) 1940 to 1968 ii) 1968 to 1991, and iii) 1992 to 2002.

2.2.2 Optimised selection

The approach of Meuwissen (1997) with the constraint on the rate of inbreeding of Grundy et al. (1998) was used to calculate the number of males and females and their mating proportions (**c**) that maximize genetic gain while constraining the rate of inbreeding to a predefined level. A vector (**g**) of predicted transmitting abilities (PTA) from the August 2003 genetic evaluation for the selection candidates was constructed for the two indices currently used in the UK: production profit index (£PIN) and profitable life index (£PLI) (MDC, 2003). £PIN is an index composed of milk, fat and protein whereas £PLI includes lifespan (based on indicator traits such as mammary traits and somatic cell count) in addition to production. The two indices were analysed to determine how important the breeding objective is when using optimised selection. The algorithm maximises genetic gain, $\mathbf{c}'\mathbf{g}$, subject to two restrictions. The first is a restriction on the rate of inbreeding while the second restriction ensures that the sum of male and female selection candidate contributions equal ½ each.

Two types of optimisations were undertaken. The first optimisation was when no constraint was placed on the contribution of selection candidates. In this situation it is assumed that there is no restriction on the reproductive capacity of either sex such that males can contribute unlimited semen and cows can contribute unlimited ova. In the second optimisation all females were selected and only male contributions were optimised.

Two groups of selection candidates were considered. In the first group the selection candidates were the parents of the top 2000 males (**Top 2000**) born in 2002 and in the second group the selection candidates were parents of the top 1000 males (**Top 1000**) born in 2002. Males were ranked on their pedigree index value for £PLI or £PIN. The pedigree index values were based on the August 2003 genetic evaluation of their parents. These males represent the result of actual selection decisions made in the previous generation and are the bulls that are available to breeding companies for potential progeny testing. Both male and female selection candidates were required to have at least four generations of complete pedigree recorded.

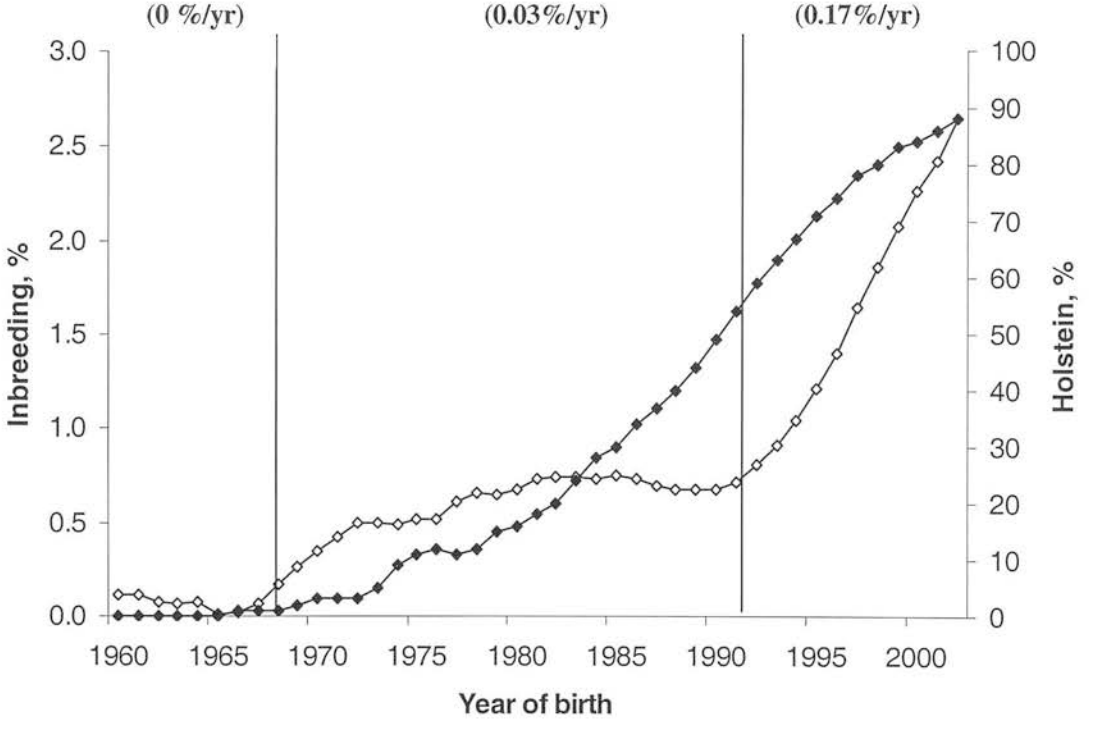
The benefits of using optimised selection were assessed by comparing the expected average pedigree index using optimised contributions to the expected average pedigree index using observed contributions at the same level of inbreeding. To do this, the observed contributions of parents were calculated based on the number of offspring born. These observed contributions were used to determine the expected pedigree index and level of inbreeding of their offspring. Optimised selection was then used for the same set of parents to calculate optimum contributions and the

expected average pedigree index of offspring. Optimised selection for the two indices (£PLI and £PIN) and two groups of selection candidates (Top 2000 and Top 1000) was investigated for five levels of constraint on inbreeding.

2.3 Results

The base population for the analysis of inbreeding was 1940. The rate of inbreeding in the population was effectively zero until 1960 so the results reported here describe the population trends since 1960. Mean inbreeding coefficients and rates of inbreeding are shown in Figure 2.1.

Figure 2.1. Average inbreeding coefficients (◊), percent Holstein (♦) and rates of inbreeding (in parentheses) for the UK dairy population.



The increase in inbreeding was non-linear in the whole period evaluated and three distinct time periods were considered in terms of the rate of inbeeding. From 1940 to 1967 there was no increase in the rate of inbreeding. There was a slight increase (0.03%/yr) from 1968 to 1991, but since 1992 inbreeding has increased at a rate of 0.17%/yr. In 2002, the average inbreeding was 2.64% for females and 3.06% for males. The number of animals inbred and the number in higher inbreeding classes has increased significantly since 1990 (Table 2.1). Currently, 98% of all males and 96% of all females are inbred to some degree compared to around 50% in 1990 (Table 2.1).

Table 2.1. Frequency of males and females by level of inbreeding (F) for animals born in 1990 and 2002.

	Males		Females	
Inbreeding %	**1990**	**2002**	**1990**	**2002**
$F = 0$	0.474	0.015	0.497	0.040
$0 < F \leq 6.25$	0.502	0.910	0.486	0.907
$6.25 < F \leq 12.5$	0.021	0.066	0.012	0.045
$12.5 < F \leq 25$	0.003	0.009	0.005	0.006
$F > 25$	0	0	0	0.002

The increase in inbreeding has coincided with an increase in the percent Holstein in the population (Figure 2.1). A slight decrease in inbreeding occurred around the late eighties and early nineties as the population of then mainly British Friesian cows were mated to imported Holstein sires. However, influential Holstein sires quickly became established in the UK and were used extensively in the early nineties. During this time the average relationship of the top 10 most widely used paternal grandsires

rose from 2% in 1987 to almost 12% in 1992 (results not shown) with some sires having 40,000 granddaughters or more entering the recorded national herd each year.

A summary of the number, mean £PLI and £PIN of male and female selection candidates is in Table 2.2. The average relationship between the males and females candidates was 6.6% and 7.3% for Top 2000 and Top 1000 respectively. For clarity of presentation, the following results relate to the analysis of Top 2000 for £PLI. Results for the other data sets are similar and are therefore not presented.

Table 2.2. Summary data for the two groups of selection candidates.

	Top 2000			Top 1000		
	No. [1]	**£PLI** [2]	**£PIN** [3]	**No.**	**£PLI**	**£PIN**
Males	173	56	54	76	64	63
Females	1642	52	51	729	65	65

[1] No. = number of male and female parents
[2] £PLI = average £PLI index scores of parents
[3] £PIN = average £PIN index scores of parents

Expected average pedigree index and inbreeding using the observed and optimum contributions are in Table 2.3. For the purpose of these results ΔF relates to the difference between the average inbreeding coefficients of the offspring compared to the average inbreeding coefficient of the parents. The expected ΔF resulting from the observed contributions was 2.0% and the expected £PLI of the offspring was £62. Optimising contributions of the selection candidates to attain the observed ΔF (i.e., $\Delta F = 2\%$) resulted in an average £PLI of £72 when male contributions only were optimised and £102 when the contributions of both sexes were optimised. This

represents an increase in genetic gain of 16% and 62% respectively over current gain when using optimised selection.

Optimised selection was successful in constraining ΔF to the predefined levels. As expected the number of animals required to have non-zero contributions increased when more severe constraints were placed on ΔF. For example, when contributions of both sexes were optimised the total number of animals required increased from 49 (25 males and 24 females) to 89 (42 males and 47 females) as ΔF was reduced from 2% to 0.1%. Similar numbers of males and females were required to meet the constraint at each level. When only male contributions were optimised, 11 sires were required at ΔF = 2% increasing to 46 when ΔF = 0.1%. Forty-four sires were required to achieve the same genetic gain as the observed (173 sires) but ΔF in the offspring was reduced ten-fold (ΔF = 0.2%). At each level of inbreeding constraint, more animals were required to achieve an optimal solution when the index under consideration was £PLI compared to £PIN. Optimisation on £PIN required 7, 6, 3 and 2 less males than £PLI for ΔF = 0.1%, 0.2%, 0.5% and 1% respectively when males only were optimised (results not shown). This is most likely due to re-ranking of the sires for the two indices. Equal numbers were selected at ΔF = 2%.

Figure 2.2 shows the relationship between optimal contributions and index scores (£PLI) of selected males for three levels of ΔF. As the constraint is relaxed fewer sires are required and there is a much stronger association between the contributions and £PLI. At the most relaxed constraint (ΔF = 2%) the individuals with the highest £PLI have the highest contributions. However, when ΔF is constrained to 0.2%, the

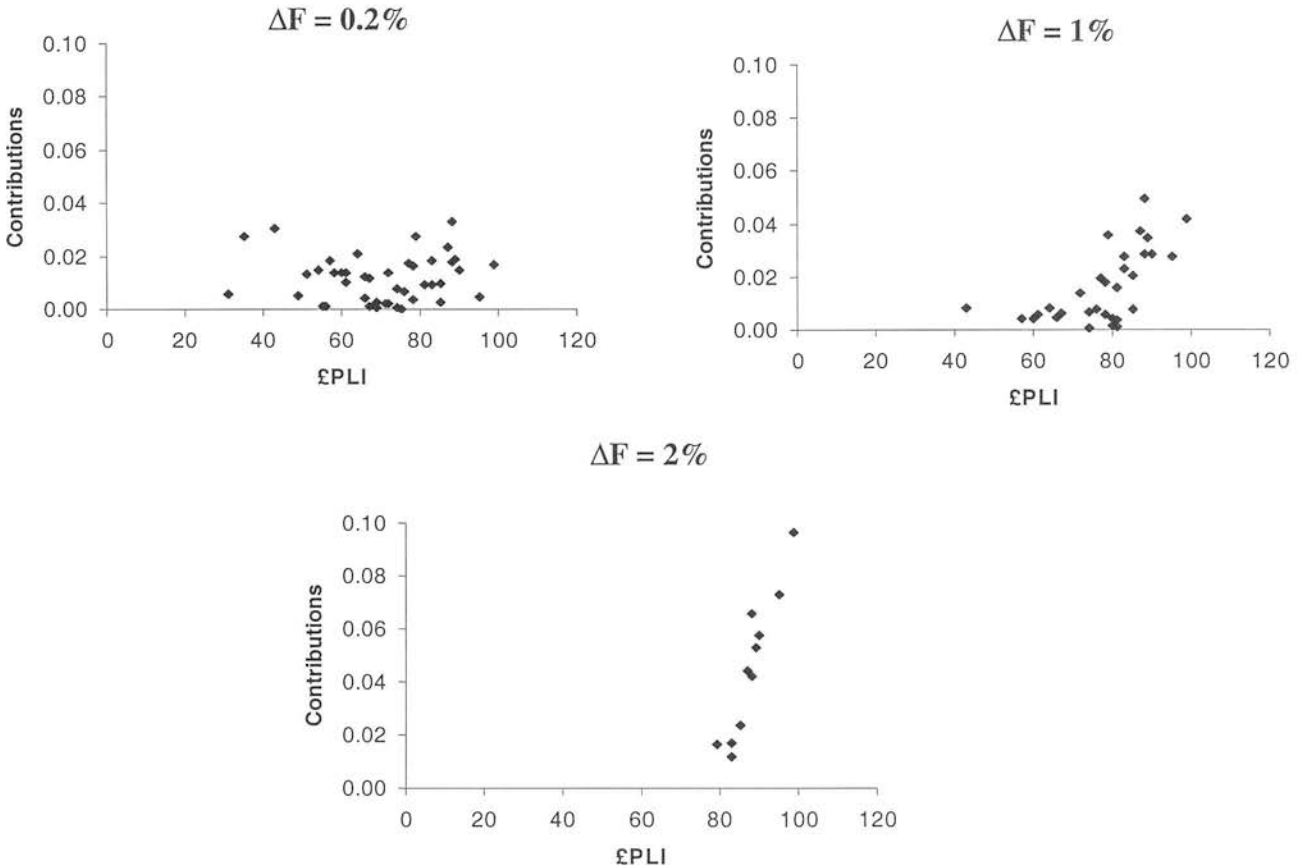
males with the highest £PLI do not necessarily have the highest contributions. Also, relaxation of the constraint led to a greater difference of contributions among selected sires.

Table 2.3. Rate of inbreeding (ΔF), expected average index score, and number of selected males and females from observed and optimised mating proportions for £PLI and Top 2000.

	Observed	Optimised Selection[1]									
		Males only					Both sexes				
ΔF (%)[2]	2.0	0.1	0.2	0.5	1.0	2.0	0.1	0.2	0.5	1.0	2.0
£PLI[3]	62	60	62	65	68	72	90	91	93	97	102
No. males[4]	173	46	44	39	31	11	42	41	39	34	25
No. females[5]	1642	1642	1642	1642	1642	1642	47	46	39	34	24

[1] Males only = male contributions optimised and female contributions fixed; Both sexes = male and female contributions optimised
[2] ΔF = difference between average inbreeding coefficient of the offspring and parents
[3] £PLI = expected mean pedigree index score in the next generation
[4,5] Number of males and females selected

Figure 2.2. Association between optimised contributions and £PLI index scores for 3 levels of constraint on inbreeding (ΔF) for selected males for Top 2000.



2.4 Discussion

The rate of inbreeding in the UK Holstein population has increased considerably in the last decade. Roughsedge et al. (1999) estimated a mean inbreeding coefficient of 0.43% for animals born in 1997 with a base year 1960. For the same year, our estimated mean inbreeding coefficient was considerably higher (1.64%). More interestingly, the rate of increase in inbreeding from 1992 to 1997 was only about 0.01%/yr (Roughsedge et al. 1999) compared to 0.17%/yr in this study. These differences may be attributable to the different base years, the use of random samples and missing pedigree information, in particular from foreign sires, in the study of Roughsedge et al. 1999. The average level of inbreeding is somewhat uninformative, as it is dependent on the definition of the base year. Since the UK population started many decades before the base year of 1940 used in this study, average inbreeding coefficients in this study are likely to be underestimated.

Another reason for the underestimation is that grade up animals with incomplete pedigrees are also included. Animals with fewer than three complete generations of pedigree information account for only about 4% of the animals born in 2002 but it is possible that some bias comes from this source as they may be inbred but assumed non-inbred.

On the other hand, the rate of inbreeding provides a more meaningful description of inbreeding in the population. Over the last decade or so, inbreeding has increased at a rate of 0.17%/yr. This equates to an increase of about 1% per generation. Slightly higher rates of inbreeding have been observed in the US (AIPL, 2003) and Canadian

(CDN, 2003) Holstein populations over the same time period. The increase in the rate of inbreeding in the early nineties could be due to a number of factors occurring in the UK dairy cattle around this time. A few very popular foreign related sires started to exert a large influence on the breed, which quickly led to an increase in the relatedness of the population. Also around this time, the individual animal model was implemented as the genetic evaluation method of choice for dairy cattle in the UK. The individual animal model utilises information from all relatives thereby increasing the co-selection of related animals, especially within 'good' families. As the large importation of North American Holstein sires in the UK continues, it is likely that the trend in inbreeding will follow that of the US and Canada but with a lag of about one generation unless measures are taken to control inbreeding.

For dairy cattle breeders one of the consequences of inbreeding will be losses due to inbreeding depression. Inbreeding depression has been shown to have a negative effect on production traits (e.g., Thompson et al. 2000; Smith et al. 1998) but it is expected to be greater in non-production traits such as fertility and disease resistance. The inclusion of health and fertility traits in national selection indices indicates the increasing importance of these traits in the breeding goal and it is necessary to examine inbreeding depression in these traits. Wall et al. (2005) found that inbreeding had a significant effect on traits such as calving interval, days to first service, non-return rate at day 56 and the number of inseminations required for a cow to become pregnant in the UK dairy population. More significantly, they found that the effect of inbreeding was more severe at higher levels of inbreeding. This study has shown that nearly 96% of the cow population are now inbred and a greater

proportion of cows are present in higher inbreeding classes than in 1990 (Table 2.1). If the current trend continues it is likely that a greater proportion of animals will be in the higher inbreeding classes leading to greater inbreeding depression for these traits.

Significant inbreeding depression suggests that the current rate of inbreeding should be controlled or reduced if further losses are to be avoided. In recent years optimisation tools have been developed to restrict the rate of inbreeding in a population. Furthermore, optimised selection has resulted in higher genetic gains at the same rates of inbreeding, or lower rates of inbreeding at the same gain when compared to truncation selection (Villanueva et al., 2004; Avendaño et al., 2003). Weigel and Lin (2002) examined the use of optimised selection in five dairy breeds in the US. Unsurprisingly, they found a reduction in genetic merit, as the constraint on inbreeding became more severe. This would be expected, as more animals with lower breeding values are required to achieve more severe constraints. However, they did not compare optimised selection with current selection to assess its ability to achieve higher genetic gains at the same level of inbreeding. The application of optimised selection will not only depend on the ability to achieve a predefined rate of inbreeding but also to increase genetic gains compared to current selection strategies. The structure of dairy cattle breeding means that long-term inbreeding will depend mostly on the relationship between the sires and dams of young AI bulls (Weigel and Lin, 2002) rather than the use of particular bulls to breed cows. In this study, we applied optimised selection to groups of selection candidates whose offspring are potential young AI bulls. Expected average pedigree index scores when the

contributions of the selected candidates were optimised were compared to the expected pedigree index scores using observed contributions at the same rate of inbreeding. For the Top 2000 males, the expected ΔF from observed parental contributions was 2% and the average £PLI was £62. At the same ΔF when only male contributions were optimised an average £PLI of £72 was achieved. The increased gain is the result of increasing the selection intensity on the sires using optimised selection. At the same ΔF only 11 sires are chosen with optimised selection compared to 173 sires originally used. A similar benefit of optimised selection can be seen when ΔF are compared at the same genetic gain. Optimised selection achieves the same average pedigree index score (£PLI = 62) as the observed genetic gain but ΔF was only 0.2%. This is ten-fold less than the observed ΔF demonstrating that current levels of gain can be achieved at lower rates of inbreeding. When contributions of both sexes were optimised the expected average pedigree index of the offspring was £102. This type of optimisation can sometimes lead to unrealistic numbers of males and females selected. However, in the situation where only a few hundred male offspring are required, it may be possible to generate the required number of offspring with the numbers of males and females selected using optimised selection for both sexes. For example, to achieve a ΔF of just 0.1%, 42 males and 47 females would be required. The expected merit of their offspring would be £90 – a 45% increase in genetic gain compared to the observed gain. With AI and the use of advanced reproductive techniques, such as *in-vitro* fertilization and embryo sexing, it would be possible to generate enough offspring to achieve these goals.

Optimised selection was successful in meeting the constraint on ΔF for all levels. In agreement with Weigel and Lin (2002), the number of animals selected increased and genetic gain decreased as the severity of the constraint on ΔF was relaxed. In order to meet the more stringent constraints the relationship between the selection candidates needs to be reduced. This means selecting more animals and allocating more non-zero contributions to animals with lower average index scores (see Figure 2.2). This has important implications in determining an acceptable rate of accumulation of inbreeding. Inbreeding is inevitable in the Holstein population, but the optimum rate of inbreeding is unknown. In terms of dairy cattle breeding the optimum rate of inbreeding might be where the differential between the gains from genetic progress and the losses due to inbreeding depression are maximized. For example, Weigel and Lin (2002) calculated an optimum level of inbreeding of 7% in the next generation for Lifetime Net Merit adjusted for inbreeding. Genetic merit decreased for levels of inbreeding > 7% as inbreeding depression had a greater impact at these increased levels. For levels less than 7%, more animals are required to meet the constraint and genetic gain is reduced.

Results from applying optimised selection could differ when multiple indices are available to choose selection candidates. In this study we choose to look at the two indices currently used for the UK dairy industry. While a high correlation exists between the two indices, the number of selected animals required to meet the constraints varied especially at more severe constraints. For example at a ΔF of 2%, 11 males were selected for both indices. However, six extra males were required to meet a constraint of 0.2% for £PLI. Inclusion of further traits such as fertility will

likely lead to a greater re-ranking of sires among the different indices and hence a change in the number of animals required to meet a specific constraint.

In the US the average relationship of a particular sire to the female population is available to breeders. This can be used by breeders to choose outcross sires if they wish to control inbreeding on an individual herd basis. However, this will likely have little impact on the overall rate of inbreeding in the population as a whole. There is also the danger is that if 'outcross' sires of high merit become available they may be heavily used in the future.

As concluded by both Avendaño et al., 2003 and Weigel and Lin (2002), the application of optimised selection requires a co-ordinated policy on the use of selected candidates and also breeding objectives. This may require the co-operation of pedigree breeders and AI organizations to ensure the correct animals and mating proportions are identified. In any case, the benefits of using optimised selection not only for controlling the rate of inbreeding but also to achieve higher genetic gains are already clear.

2.5 Conclusions

While inbreeding levels in the UK dairy population are not at alarming levels yet, it appears likely that the current rapid rise in inbreeding will continue. The increase in inbreeding will lead to greater inbreeding depression and possibly increased prevalence and numbers of genetic recessive disorders in the population. Routine estimation of rates of inbreeding and inbreeding depression, especially for non-

production traits, should be conducted to monitor the effects of inbreeding. The use of optimised selection should provide a useful tool to manage the rate of accumulation of inbreeding in the population. More importantly however, is that use of optimised selection can increase genetic gains for a given rate of inbreeding or attain similar genetic gains at much lower rates of inbreeding when compared to current selection strategies.

CHAPTER THREE

CUMULATIVE DISCOUNTED EXPRESSIONS OF SIRE GENOTYPES FOR THE CVM AND β-CASEIN LOCI IN COMMERCIAL DAIRY HERDS

3.1 Introduction

Dairy cattle genetics is a dynamic industry with breeding companies and breeders constantly facing new challenges. The emergence of molecular genetics has presented the industry with new technologies that can be used in conjunction with traditional selection methods to enhance genetic progress. For example, DNA tests for the detection of genetic defects are widely used (Dekkers, 2004). More recently, DNA based tests have been developed whereby an animal with a favourable genotype for a specific trait (e.g. feed intake, marbling, reproduction, milk production) can be selected to increase the rate of genetic gain for that trait (Dekkers, 2004).

Genetic defects are often uncovered retrospectively. For instance, Complex Vertebral Malformation (CVM), a recessive defect resulting in early abortion or stillbirths was discovered only recently (Agerholm et al. 2001) but its origin can be traced back to a bull used several decades ago. Approximately 50% of embryos are aborted before day 150 (Nielsen et al., 2003) therefore the effects of CVM are likely to result in longer calving intervals and higher culling than actual dead calves. Breeding companies often continue to market sires with a single copy of a deleterious allele, especially those of high genetic merit. Currently, 16 out of the Top 100 Holstein sires for production in the UK are CVM carriers (Holstein UK, 2004). Often breeders continue to use these sires, perhaps without due consideration of the effects that this may have in the herd. In order to make more informed decisions some questions should be answered first. For example, what are the costs and benefits of using carrier sires in a herd over time? Also, should

the cost per unit semen of a carrier sire be less than that of a sire with no copies of the defective allele but with equal genetic merit for a particular breeding objective?

The assessment of the costs and benefits of using commercially available DNA based tests to aid selection for traits of economic importance is also imperative before employing them as a tool in selection. One such test is for the A2 variant of β-casein. Currently a premium is being paid for homozygous A2A2 milk in New Zealand and Australia.

Discounted gene-flow techniques are useful to assess the benefits of using genotypic information when making breeding decisions (e.g. Amer, 1999; Wood et al., 2004). These techniques account for the fact that benefits or costs realised from genetic expressions in future years have lower effective value than those incurred immediately.

The objective of this study was to evaluate the use of sires carrying specific alleles of interest in commercial dairy herds by applying discounted gene-flow techniques. Two applications of using genotypic information at the commercial herd level were used to illustrate the method. The first application (case study I) dealt with the commercial value of using sires that are carriers of a deleterious recessive allele. The CVM allele was used as an example. The second application (case study II) dealt with the value of sires that are carriers of a specific beneficial allele. The A2 variant of the β-casein gene was used as an example.

3.2 Materials and Methods

3.2.1 Gene flow

In a dairy herd the genes of a particular sire are expressed not only in his daughters but also in all his descendants. The cumulative expressions from using a sire of a particular genotype in the herd depends on a number of factors including the age distribution of cows in the herd, the frequencies of cow genotypes, the number of years a sire is used and the planning horizon.

Let $p_{i,t}$ be the proportion of sires of genotype i at year t. These proportions were specified in advance for each of the genotypes and for each of the years $t = 0$ to $t = h - 1$ where h is the planning horizon beyond which impacts of the allele of interest are ignored. Also, let s_l be the proportion of breeding cows of age l, and $k_{i,l,t}$ the proportion of cows of genotype i, age l at time t. For this study the minimum breeding age was one and the maximum breeding age was seven (i.e. l= 1 to 7). Therefore the proportion of cows of genotype i mated at time t ($c_{i,t}$) in the herd can be computed as:

$$c_{i,t} = \sum_{l=1}^{7} s_l \, k_{i,l,t} \qquad [1]$$

The proportion of offspring of genotype i at time of birth t ($o_{i,t}$), under the assumption of random mating and no differential effect of the alleles on fitness, can be calculated from:

$$o_{i,t} = \sum_{j=1}^{n} \sum_{k=1}^{n} P(o_{i,t} \mid S_{t-1}, D_{t-1}) \, p_{j,t-1} \, c_{k,t-1} \qquad [2]$$

where n is the number of genotypes for the gene of interest and $P(o_{i,t}|S_{t-1},D_{t-1})$ is the probability of an offspring of genotype i at time t, given a sire of genotype S and a dam of genotype D at time of mating $(t-1)$, and can be calculated from simple Mendelian rules. For example, for a gene with two alleles A and a, $P(o_{i,t} = AA|S_{t-1}= AA, D_{t-1}= AA) = 1$ and $P(o_{i,t} =Aa|S_{t-1}= Aa, D_{t-1}= Aa) = 0.5$ and so on.

From equation [2] it is clear that the herd genotypes $(c_{i,t})$ and offspring genotypes $(o_{i,t})$ are inter-dependent and must be computed recursively. At $t = 0$, the proportion of cows of each genotype and age $(k_{i,l,0})$ was specified assuming Hardy-Weinberg equilibrium in the herd. The initial distribution of genotypes was assumed the same across age groups. The proportions of cows one year old (s_1) and older in year $t = 1$ (s_{2-7}) were computed from the proportions in year $t = 0$ lagged by 1 year of age. Breeding cow genotypes in year t were computed using equation [1]. From this, and the proportion of bulls used at time t, new offspring genotype proportions can be computed for year $t + 1$ using equation [2].

In this study we are interested in the consequences (in terms of costs and benefits) of increasing the proportion of homozygous recessive animals as a result of using sires of a particular genotype (see case studies below). The cases considered involved biallelic loci. The cumulative expressions of the sires' genes over the planning horizon need to be discounted back to the time when the sire was first used to account for the delays in the expression of genes.

Let **q** be a discounting vector with elements

$$q_i = \left(\frac{1}{1+r}\right)^{i-1}$$

where $i = 1$ to h and r is the discount rate. Four different discount rates were considered.

The cumulative discounted expressions (*CDE*) of genotype i for calves at birth (required for case study I) were calculated by multiplying the proportion of homozygous animals for the recessive allele in year t, by the corresponding element of **q** and summed over all years:

$$CDE_i = \sum_{t=1}^{h} o_{i,t} q_t$$

The *CDE* for the expression of genotype i for cows in any lactation (required for case study II) was calculated as:

$$CDE_i = \sum_{t=1}^{h} c_{i,t} q_t$$

The *CDE* were then multiplied by the economic values of specific genotypes of interest to determine the value of using particular sire genotypes.

3.2.2 Effect on semen price

Given the risks associated with using carrier sires it may be appropriate to pay less for semen from these sires. Likewise, the benefit accruing from using A2A2 sires could

result in a premium being paid for such semen. A reduction or premium (£) per unit semen was calculated as:

$$\text{Semen Reduction/Premium} = \frac{Cost/Benefit\ of\ the\ gene \times CDE}{Units\ of\ semen\ used \times Services\ per\ conception}$$

Where the cost of using CVM carrier sires was assumed to be the cost of a dead calf and the benefit of using A2A2 sires was the value of an A2A2 cow versus a non-A2A2 cow. The value assumed for the number of services per conception was 1.7, which corresponds to the mean value, found in UK cows (Wall et al. 2003) and the cost of a recessive case is given below.

3.2.3 Case Study I

This case study examines the costs associated with using a sire that is a carrier for a lethal recessive genetic defect such as CVM. The following parameters would influence the impact of using a CVM carrier sire on a herd: 1) the frequency of carrier cows in the herd; 2) the proportion of carrier sires used each year; 3) the number of years that carrier sires are used, and 4) the cost of each recessive case. Due to the nature of CVM it was assumed that all recessive animals (aa) die. Because of this it is necessary to adjust the proportions of the homozygous normal and heterozygous animals to ensure that the genotype proportions sum to one. This was done by dividing the proportion of homozygous normal or heterozygous by one minus the proportion of recessives. Carrier sires were assumed to be used for either one or three years and different proportions (20%, 50% and 100%) of carrier sires were considered. Three years was considered as

the maximum to avoid the risk of excessive mortality as the frequency of CVM increased. The initial frequency of CVM carrier cows in the herd was 0, 0.05, 0.1, or 0.2.

3.2.3.1 Cost of a recessive CVM case

There will be several costs incurred as a result of a cow carrying a calf homozygous for the deleterious allele such as veterinary costs, culling and replacement costs and the calf loss. Nielsen et al. (2003) found that not all cows retain a CVM fetus for the entire gestation period. They calculated that 29% of carrier cows would abort before d100, 45% before d150 and 77% before d260. It is likely that the cows aborting after d150 will be culled, while those that abort earlier will be retained in the herd. Based on this, we assume for this study that 50% of carrier cows are culled, and 50% are retained. The value of an involuntarily culled cow depends on the age of the animal, and the current market value of a culled cow. The average cull value for cows is approximately £300 (Stott et al., 2005). Allowing a cost of £85 for veterinary expenses and labor, £200 for mean calf value and £600 for replacement costs (excluding the value of the culled cow), the approximate cost of a case of CVM resulting in a cow being involuntarily culled is £585. For a cow averaging 6,000 liters/year the cost of a delay in conception when conception occurs after 150d is estimated at £253 (Esslemont et al., 2000). Assuming that we can expect 50% of cows to be culled and 50% to survive the average cost of a CVM case would be £419 ((253+585)/2).

3.2.4 Case study II

The second case study investigated the value of using sires genotyped for the A2 variant of the β-casein gene. Premiums are paid for milk that is homozygous for the A2 allele and therefore a commercial herd manager will be interested in the returns from using A2A2 sires, and in the length of time taken to obtain a homozygous A2A2 herd. Only milk from cows with two copies of the A2 allele is desired and so are we only interested in A2A2 animals. The initial proportion of A2A2 cows in the herd was 0, 0.0225, 0.25, and 0.7225, which, assuming Hardy-Weinberg equilibrium, correspond to an initial allele frequency (f_{A2}) equal to 0, 0.15, 0.5, and 0.85. These values were chosen arbitrarily to represent a zero, low, intermediate and high initial allele frequency in the population. The *CDE* of using either A2A2 or A1A2 sires over the planning horizon were obtained as described above. The *CDE* were then multiplied by the economic value of A2 milk (see below) to calculate the potential value from using sires carrying the A2 allele.

3.2.4.1 Value of A2A2 milk

Currently New Zealand and Australia are the only countries paying a premium for A2A2 milk. In New Zealand a 4-pence per litre premium is paid for A2 milk. If, on average, a cow produces 4,000 litres of milk per year the value of an A2A2 cow is approximately £160 more than a non-A2A2 cow. It is assumed that A2A2 cows can be milked separately from other cows as mixing of their milk with that of A1 carrier cows eliminates any commercial premium.

3.3 Results

For both case studies we were interested in the homozygous (aa or A2A2) animals produced as a result of using sires of a particular genotype over a particular planning horizon. The planning horizon will be the number of years a particular sire genotype is used plus the number of years until the genetic influence of descendents becomes negligible. For example, in case study I, carrier sires were used for a maximum of three years, therefore the results refer to a planning horizon of 11 years as all effects of using these sires were minimal after this.

3.3.1 Case Study I

The number of dead calves (*aa*), rounded to the next highest integer, expected for the situations investigated are in Table 3.1. No dead calves are expected when there are no carrier cows in the herd and carrier sires are used for one year only. When carrier sires are used for three years and there are no carriers in the herd initially, dead calves begin to appear as heterozygous cows are mated to carrier sires in year 3. As expected, the number of dead calves increased with the proportion of carrier sires used and with increasing carrier cow frequency.

Table 3.1. Number of dead calves expected in a 100-cow herd when varying proportions of CVM carrier sires (CS, in %) are used for one or three years with different initial carrier cow frequencies.

	Carrier sires used for 1 year			Carrier sires used for 3 year		
Carrier Cow Freq	**CS=20**	**CS=50**	**CS=100**	**CS=20**	**CS=50**	**CS=100**
0.00	0	0	0	1	1	4
0.05	1	1	2	3	4	9
0.10	1	2	3	3	6	12
0.20	1	3	5	3	9	18

The *CDE* resulting from using varying proportions of carrier sires in a herd with different carrier frequencies are shown in Table 3.2. For this case study the *CDE* represents the discounted equivalent number of dead calves which increases with the proportion of carrier sires used. When carrier sires are used for one year only the *CDE* doubled with a doubling of the cow carrier frequency in the herd. A linear relationship between the number of *CDE* and the proportion of carrier sires used also exists when carrier sires are used for three years. Higher discount rates lead to decreased expressions. Proportionally this reduction was highest when the carrier cow frequency was lowest. For example when the carrier frequency was zero the reduction in expressions was 33% between a discount rate of five and 20% but when the frequency in the herd was 0.2 the reduction was 24%.

Table 3.2. Cumulative discounted expressions as result of using different proportions of CVM carrier sires (CS, in %) over one or three years in a 100-cow herds with different carrier cow frequencies for different discount rates, and a planning horizon of 20 years.

Carrier Cow Freq	Discount rate	Carrier sires used for 1 year			Carrier sires used for 3 year		
		CS=20	CS=50	CS=100	CS=20	CS=50	CS=100
0.00	0.05	0.00	0.00	0.00	0.13	0.81	3.24
	0.07	0.00	0.00	0.00	0.12	0.77	3.06
	0.10	0.00	0.00	0.00	0.11	0.70	2.82
	0.20	0.00	0.00	0.00	0.09	0.54	2.17
0.05	0.05	0.24	0.60	1.19	0.77	2.40	6.36
	0.07	0.23	0.58	1.17	0.74	2.30	6.07
	0.10	0.23	0.57	1.14	0.70	2.16	5.68
	0.20	0.21	0.52	1.04	0.59	1.78	4.60
0.10	0.05	0.48	1.19	2.38	1.41	3.98	9.48
	0.07	0.47	1.17	2.34	1.36	3.83	9.08
	0.10	0.46	1.14	2.27	1.29	3.61	8.54
	0.20	0.42	1.04	2.08	1.09	3.02	7.06
0.20	0.05	0.95	2.38	4.76	2.70	7.16	15.73
	0.07	0.94	2.34	4.67	2.60	6.89	15.12
	0.10	0.91	2.27	4.55	2.47	6.52	14.27
	0.20	0.83	2.08	4.17	2.09	5.50	11.95

A reduction in price for a unit of carrier semen used was calculated to determine how much less should be paid for this semen versus semen from a non-CVM carrier sire of equal genetic merit (Table 3.3).

Table 3.3. Effect of the proportion of carrier sires used (CS, in %), number of years in which carrier sires are used, and initial carrier cow frequency on the semen reduction required (£ per CVM straw used) when the cost of dead calf is £419 and the discount rate is 0.07

	Carrier sires used for 1 year			Carrier sires used for 3 years		
Carrier Cow Freq	CS=20	CS=50	CS=100	CS=20	CS=50	CS=100
0.00	0.0	0.0	0.0	0.5	1.3	2.5
0.05	2.8	2.9	2.9	3.1	3.8	5.0
0.10	5.8	5.8	5.8	5.6	6.3	7.5
0.20	11.6	11.6	11.6	10.7	11.4	12.5

For a given carrier cow frequency there was practically no difference in the reduction in price required for different proportions of carrier sires used for only one year. Similar to the pattern observed for the number of expressions, the reduction required doubled when cow herd frequency doubled. The reduction in price required increased when carrier sires were used for a period of three years as both the amount of carrier sires used increased, and as the frequency of carrier cows in the herd increased. In proportion, the reduction in price when using more carrier sires was greatest at the lowest cow herd frequency. A 64% greater reduction would be required to breakeven when using all carrier sires where the cow frequency is 0.05 compared to just 16% when the cow frequency is 0.2.

3.3.2 Case Study II

The *CDE* from using sires homozygous for the A2 variant of β-casein are shown in Table 3.4. As expected the number of *CDE* increased when the number of years of using homozygous recessive (A2A2) sires increased and also when the initial frequency of the herd increased. When the initial frequency of A2A2 cows was zero there was no benefit from using A2A2 sires for just one year.

Table 3.4. Cumulative discounted expressions as a result of using A2A2 sires for a different number of years in a 100-cow herd with different initial cow frequencies assuming different discount rates and a planning horizon of 20 years.

		No. years A2A2 sires used		
Initial Cow Freq (A2A2)		**1**	**5**	**10**
0.0000	0.05	0	323	528
	0.07	0	303	495
	0.10	0	277	451
	0.20	0	209	339
0.0225	0.05	13	394	610
	0.07	12	369	570
	0.10	11	336	520
	0.20	8	254	391
0.2500	0.05	42	557	800
	0.07	39	522	750
	0.10	36	475	682
	0.20	27	357	513
0.7225	0.05	71	720	991
	0.07	67	674	929
	0.10	60	614	845
	0.20	45	461	633

The increase in *CDE* when using A2A2 sires for 10 instead of for five years was greater at lower cow frequencies. When the initial frequency of *A2A2* cows in the herd was zero there was a 63% increase in CDE when *A2A2* sires were used for 10 versus five years. The increase was 54%, 43% and 37% for initial cow frequencies of 0.0225, 0.2500 and 0.7225, respectively. As in the previous case study, the number of CDE decreased as the discount rate increased.

The premium per unit semen was calculated when A2A2 sires were used for one, five or 10 years and is shown in Table 3.5. This could also be thought of as the reduction that should be paid for non-A2A2 semen. Not surprisingly, the value of A2A2 semen was considerable when used for five or 10 years compared to just using it for one year.

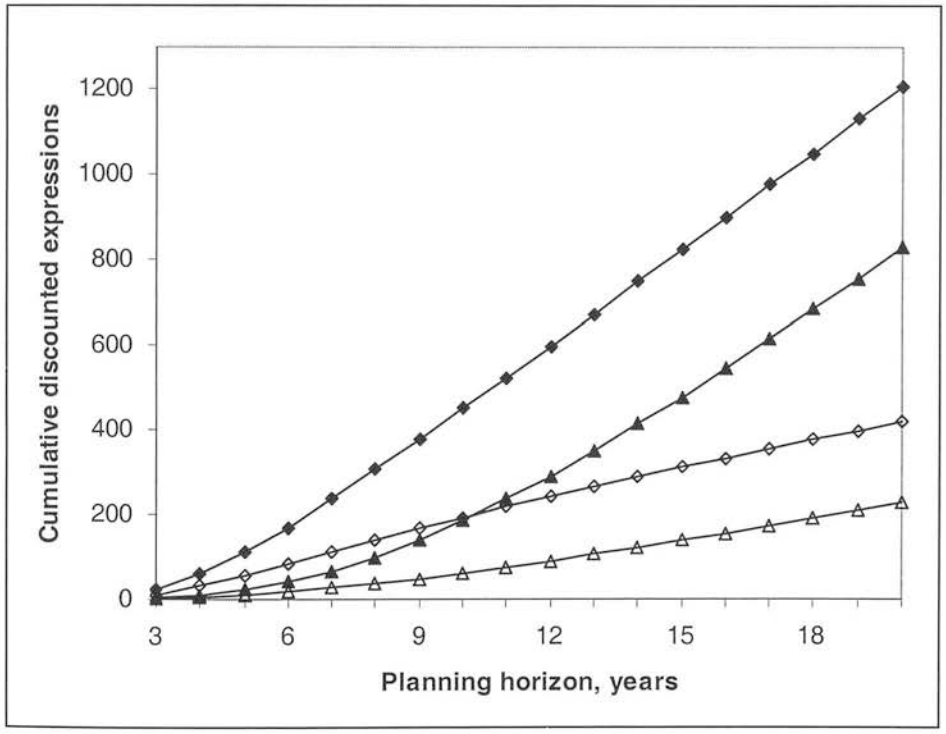
Table 3.5. Effect of the number of years A2A2 semen is used, and initial carrier cow frequency on the semen premium (£ per A2A2 straw used) when the value of an A2A2 cow is £160 more per lactation than a non-A2A2 cow and the discount rate is 0.2.

	Years A2A2 Sires used		
Carrier Cow Freq	**1**	**5**	**10**
0	0	3.93	3.20
0.0225	0.75	4.78	3.68
0.25	2.50	6.75	4.82
0.7225	4.25	8.67	5.96

Figure 3.1 compares the *CDE* over the planning horizon when all sires used were either A2A2 or A1A2 for two initial starting frequencies of A2A2 cows in the herd. When only A2A2 sires were used and the initial A2A2 frequency in the herd was 0.7225 ($f_{A2} = 0.85$,

where f_{A2} is the frequency of the A2 allele) there was a linear increase in *CDE* over the planning horizon. A similar pattern emerges when only A1A2 sires were used but the number of *CDE* was three times less than when A2A2 sires were used. When the initial frequency of A2A2 cows in the herd was 0.0225 ($f_{A2} = 0.15$), the rate of increase in the *CDE* was slow until around year 12 after which the *CDE* increased linearly. After 20 years the *CDE* was 828 compared to 1,209 when the initial frequency was 0.7225.

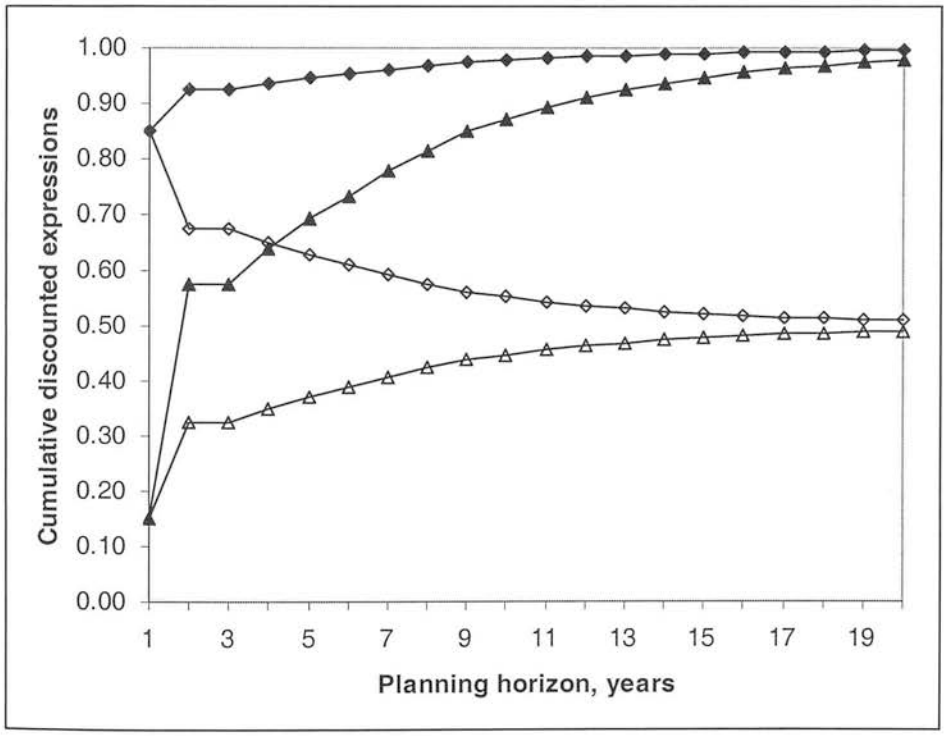
Figure 3.1. Cumulative discounted expressions over the planning horizon when either all A2A2 (♦, ▲) or all A1A2 (◊, Δ) sires are used and the initial frequency of the A2 allele in the herd is 0.15 (▲, Δ) or 0.85 (♦,◊).



The evolution of the allelic frequency for A2 over the planning horizon is shown in Figure 3.2. When the initial frequency in the herd was high and A2A2 sires were used

continuously the frequency in the herd increased towards one. The greatest increases occurred in the earlier years. However, when A1A2 sires were used the frequency of the A2 allele decreased towards 0.5. The greatest decrease occurs the year after the A1A2 sires started to be used. When only A2A2 sires were used and the initial frequency was low the greatest increase occurred in the first years and thereafter the frequency increased steadily towards one. A frequency of 0.9 was reached after 12 years. Using A1A2 sires increased the frequency of the allele A2 to about 0.5.

Figure 3.2. Evolution of the A2 allele frequency in the herd when either all A2A2 (♦, ▲) or all A1A2 (◊, Δ) sires are used and the initial frequency of the A2 allele in the herd is 0.15 (▲, Δ) or 0.85 (♦,◊).



The value of using an A2A2 sire can be calculated from the *CDE* and the economic value of A2A2 milk. For example, the *CDE* of using an A2A2 sire for five years, when 25% of the herd is A2A2 (approximate proportion of A2A2 cows in New Zealand) at a discount rate of 20% is 357 (Table 3.4). Assuming that the value of milk from A2A2 cows is £160 more than non-A2 milk, and a planning horizon of 20 years the benefit of using A2A2 sires over non-A2A2 sires that are otherwise equal in merit, is 357 × £160 giving a economic return of £57,120.

3.4 Discussion

This study demonstrates the complexity of the economic implications of using information for a single genetic locus with non-additive economic effects when selecting bulls to use to mate cows in a commercial dairy herd. There is extensive literature dealing with the economic implications of selection decisions in relation to expression of differences in estimated breeding values (e.g. Miglior, 2005; VanRaden, 2004). These approaches involve tracing the multiple expressions of the sires' genes for a range of traits in their descendants at specific ages. For a single locus with non-additive economic effects, the results here demonstrate that further account must be taken of the starting genotype frequencies in the herd, and the duration of use of bulls of specified genotypes.

Despite the existence of numerous genetic defects in dairy cattle, little research has been done on the effects of using a carrier sire of such defects in a cow herd. Often superior sires that are carriers will continue to be marketed. Advice on whether to use carrier

sires is often conflicting and is further complicated by the opposing policies of breeding companies on the sale of semen from carrier sires. It is generally assumed that over time a genetic defect will be eliminated. However, if carriers of a specific genetic defect have high genetic merit for other economically important traits, then the value of using a carrier sire could increase dramatically. To date, very little literature exists on the possible links between the CVM status and performance in other economically important traits in dairy cattle. Hansen et al. (2004) found a low impact of CVM on the genetic trend for stillbirth, but this may be explained by the large percentage (77%) of CVM homozygous fetuses that are aborted before d260 of gestation (Nielsen et al., 2003). In this study we assumed no difference in genetic merit between CVM carrier sire and a non-CVM carrier sires. However, it is not inconceivable that a favourable relationship may exist between a carrier genotype and some performance traits. For example, in pigs, a favourable effect of the halothane gene on lean meat has meant it has remained in the population, despite resulting in pigs more susceptible to stress and sudden death (Nicholas, 1996).

Here we have looked at the implications of using sires that carry one copy of the allele responsible for CVM. The cumulative discounted expressions were calculated for situations where the proportion of carrier sires, the duration of carrier sire use, the initial frequency of carriers in the cow herd, and the discount rate varied. When the initial cow carrier frequency is zero, there is no loss accrued from using carrier sires for one year only. When carrier sires are used for three years the *CDE* are greater than three times the one year values as the frequency of carrier cows will be increased as a result of using a

carrier sire initially and also because a greater proportion of carrier sires are mated to carrier females in years two and three, resulting in more expressions. In terms of the actual number of dead calves, if the frequency of carrier cows in a herd were unknown it appears that the best strategy would be to keep carrier use at or below 20%. For the carrier cow frequencies investigated, there was no difference in the number of dead calves expected when 20% carrier sires are used. However, the number of dead calves increases with carrier cow frequency when 50% or 100% of carrier sires are used.

Using a carrier sire involves a certain amount of financial risk, therefore a breeder should be able to purchase semen from a carrier sire for less than a non-carrier sire of equal genetic merit. In general a greater reduction in the cost of CVM semen is required with increased carrier sire use and cow frequency. It is unlikely that the frequency of carrier cows in a herd is zero, therefore it is evident from these results that many breeders will require a reduction in semen price before using carrier sires of a known genetic defect when compared to non-carrier sires of equal genetic merit.

Most studies investigating the benefit of using genes of known effect or markers linked to them in dairy breeding programs have focused on their use in nucleus breeding herds (e.g., Meuwissen and van Arendonk, 1992; Schrooten et al., 2005). However, DNA based tests are now available to commercial breeders to allow them to select for a gene influencing a specific trait. Apart from parentage checking and genetic defect testing, DNA tests are available for genes affecting quantitative traits such as milk yield and composition (β-lactoglobulin, β and κ-casein, DGAT1, BGHR) and feed intake (leptin).

Alleles of β-lactoglobulin were shown to increase cheese yield without affecting quality or taste while the B allele of κ-casein has favourable effects on milking processing properties. Properties such as these are likely to realise a premium from milk processing companies, and therefore increase the profitability of an enterprise. Recent studies have suggested a link between the A2 variant of β-casein and favourable health benefits (e.g. Tailford et al., 2003; Laugesen and Elliot, 2003). If such a relationship exists then it might be desirable for a producer to increase the frequency of A2 cows in the herd as the value of milk from these animals may be significantly increased. Even with a high discount rate of 20% (i.e. where more preference is given to earlier expressions) we have shown that there is considerable benefit from using A2A2 sires to increase the frequency of A2A2 cows when a premium is paid for milk from these cows. The higher the initial allele frequency the greater the benefit as it takes six to seven years to build up the proportion of A2A2 cows in the herd when the initial frequency is low. Continual use of A2A2 sires will increase the proportion of A2A2 cows in the herd but the time taken to have a pure A2A2 herd will depend on the initial frequency in the cow herd. Even when the A2 allele is at a low frequency (0.15) the use of A2A2 sires for one year can increase the proportion of A2A2 cows in the herd to > 50% after three years. A more rapid but expensive method of increasing the proportion of A2A2 cows would necessitate genotyping all the cows in the herd. These genotypes can then be used in culling and selection decisions to maximize the proportion of A2A2 replacements.

In these studies we were only interested in one particular offspring genotype. The same approach can be used to determine the value of sires in a situation where heterozygous descendants also contribute to the value of a sire. The *CDE* from this scenario will be

greater than those obtained here as heterozygous animals will contribute to the expressions in addition to the homozygous animals. The method can be used to calculate returns from two or more unlinked genes or quantitative trait loci (QTL) by calculating the returns independently for each gene and summing them together. Similarly, the value of a pleiotropic QTL can be obtained by calculating the *CDE* for each trait separately, multiplying by the economic value of the QTL for each trait and summing them together.

Usually indexes are available which quantify economic consequences of selecting bulls differing in genetic merit for polygenes. Depending on the base of expression of the index a translation may be required to enable a meaningful tradeoff to be made between polygenic merit and the value of gene expression as calculated here.

3.5 Conclusions

This study quantifies the costs (in case study I) and benefits (in case study II) for two single genetic loci that have received some attention by dairy breeders in recent times. It has demonstrated how a recursive set of calculations can be used to quantify expected changes in genotype frequencies in a dairy cow herd over time as a result of using sires of a specified genotype. The commercial implications of changes in gene frequencies have been demonstrated for a deleterious/lethal recessive gene (CVM) and for an economically favourable allele affecting milk composition (A2). The results show how the starting gene frequency in the herd, and the proportion and duration of use of sires of particular genotypes are critical when evaluating the economic implications of the use of single genes in commercial dairy herds.

CHAPTER FOUR

MAINTENANCE OF DELETERIOUS ALLELES FOR FITNESS AS A CONSEQUENCE OF ARTIFICIAL SELECTION ON PRODUCTION TRAITS

4.1 Introduction

In domestic livestock species, improving disease traits has become increasingly important due to the need to reduce the costs of production and to satisfy consumer concerns about the health and welfare of animals. There is thus an interest in increasing the frequency of disease resistance alleles and eliminating those causing disease from the population. Examples include reducing mastitis and lameness in dairy cattle, parasite and scrapie susceptibility in sheep, and metabolic disorders such as ascites in chickens.

In order to evaluate the benefits of including disease traits in the breeding objective it is necessary to know their genetic relationships with other traits of importance such as production traits. Traditionally, this has been done through the estimation of (additive polygenic) genetic correlations, using animal models. It is generally accepted that if the estimated correlation between production and disease resistance/susceptibility is zero, improvement in production traits can be accomplished without adversely affecting the disease trait. In such a situation it might be expected that natural selection would act to eliminate the alleles that adversely affect fitness.

Quantitative trait loci (QTL) mapping experiments have provided a means for looking at the underlying genetic architecture of quantitative traits. Due to the readily available data, many QTL detection experiments have focused on production traits. Nonetheless, QTL for traits such as disease resistance or susceptibility may offer more benefits, as traditional selection methods are less efficient at improving these traits, because their

heritabilities are generally low and the disease needs to actually occur in the population with a high enough frequency to allow efficient selection against it. If QTL affecting disease traits are found, selection could be carried out to reduce disease susceptibility, without the need of the disease occurring in the population at a given point in time. Mapping studies aimed at finding QTL for these low heritability traits could easily have production data available, allowing the search for linked or pleiotropic QTL. This will be important if the QTL information is to be used subsequently in marker assisted selection programs.

One particular study looking at pleiotropic QTL is that of Navarro et al. (2006a). A segregation analysis in a commercial poultry population revealed a QTL segregating at intermediate frequencies, which had negative effects on ascites resistance. Furthermore they showed this QTL had positive effects on production (weight and fleshing score), despite the estimated additive genetic correlation between the production and an indicator for ascites resistance disease traits being zero, assuming a polygenic model (Navarro, 2006b). According to the estimates, the QTL had a dominant effect on ascites resistance and an overdominant effect on production. This mode of action might explain why a gene (or two or more tightly linked genes) with negative effects on a fitness trait (ascites) is still segregating at intermediate frequencies in populations where artificial selection is applied to production traits as occurs in commercial livestock populations. Even if disease resistance were included in the breeding objective, such a mode of action would make it difficult to alter the frequency of the ascites resistance alleles by

conventional selection based on phenotypes and ignoring information on genetic markers.

The objective of this study was to investigate the effect of artificial selection for improving production traits on the evolution of the frequency of a pleiotropic QTL affecting both production and disease susceptibility. Various modes of action and amounts of variation accounted for by the QTL were considered.

4.2 Materials and Methods

Stochastic simulations were used to model artificial selection on a production-type trait affected by a QTL that is also affecting a disease-type trait. Animals for which the phenotype for disease susceptibility exceeded a given threshold were culled (i.e., natural selection was assumed to be acting on the disease trait). The expected changes in allele frequencies for the QTL under different genetic models were monitored. A total of 100 replicates were run for each simulation.

4.2.1 Genetic model

The two traits were assumed to be controlled by additive polygenes plus a biallelic pleiotropic QTL (alleles A and B). It was assumed that the A allele increased production but also increased susceptibility to disease and the B allele decreased production and reduced susceptibility to disease. The total genetic value of an individual i for each trait was $g_i = v_i + u_i$, where v_i is the genotypic value due to the QTL, and u_i is the polygenic value. The polygenic heritabilities (h^2) were 0.5 and 0.1 for production and disease

susceptibility, respectively and the polygenic correlation between the two traits was zero. The genotypic values due to the QTL were a, d and $-a$ (for individuals with genotype AA, AB and BB, respectively), where a is the additive effect defined as the half the difference between the two homozygotes, and d is the dominance effect defined as the difference between the heterozygote and the average of the two homozygotes (Falconer and Mackay, 1996). The additive variance explained by the QTL in the base generation was $\sigma_a^2 = 2pq\alpha^2$ where p is the initial frequency of the A allele, $q = (1-p)$ and $\alpha = [a + d(q-p)]$ (Falconer and MacKay, 1996). The dominance variance was $\sigma_d^2 = (2pqd)^2$.

Models differing in the values for a and d for the production trait and in p were considered. In all scenarios, the genetic mode of action of the QTL for disease susceptibility was dominant. For this trait, the genotypic values for the QTL were 0.9, –0.9 and –0.9 for genotypes AA, AB and BB, respectively. This means that AA animals which have a QTL genotypic value of 0.9 are susceptible to the disease and AB and BB animals, with a QTL genotypic value of –0.9, are resistant to it. For the production trait, additive, dominant or overdominant modes of action of the QTL were considered. The specific models assumed are given in Table 4.1. In most scenarios, the initial frequency of the A allele was 0.5, but situations where the initial frequency of A was 0.9 and 0.1 were also considered.

Table 4.1. Summary of the additive (a) and dominant values (d), corresponding genotypic values and percentage of the total variation (% Gen Var) accounted for by the QTL for production and disease susceptibility.

	Genotypic Values			
Model	**A[a]A**	**AB[b]**	**BB**	**% Gen Var**
Production				
$a1$=0.2, $d1$=0.0	0.2	0.0	-0.2	4%
$a1$=0.3, $d1$=0.0	0.3	0.0	-0.3	8%
$a1$=0.5, $d1$=0.0	0.5	0.0	-0.5	20%
$a1$=0.2, $d1$=0.2	0.2	0.2	-0.2	6%
$a1$=0.3, $d1$=0.3	0.3	0.3	-0.3	12%
$a1$=0.5, $d1$=0.5	0.5	0.5	-0.5	27%
$a1$=0.0, $d1$=0.9	0.0	0.9	0.0	28%
$a1$=0.2, $d1$=0.9	0.2	0.9	-0.2	31%
$a1$=0.5, $d1$=0.9	0.5	0.5	-0.5	27%
Disease Susceptibility				
$a2$=0.9, $d2$=0.9	0.9	-0.9	-0.9	85%

[a] Allele 'A' increases production and disease susceptibility
[b] Allele 'B' decreases production and disease susceptibility

4.2.2 Simulation of the population

One-hundred and twenty individuals (60 males and 60 females) were simulated in the base generation (t = 0). Generation 1 (t = 1) was obtained from matings of individuals selected at t = 0. For both traits, the phenotypic value for an individual i was obtained by adding the total genetic value (g_i) to an environmental component (e_i). Both u_i and e_i were obtained from normal distributions with mean zero and variances $\sigma^2_{u_j}$ and $\sigma^2_{e_j}$, respectively, where j = 1 (production) or 2 (disease susceptibility). The polygenic and environmental variances summed to one for each trait.

For generations $t > 0$ the polygenic value of the offspring was obtained by adding a random Mendelian sampling term to the average of the polygenic effects of their parents. The Mendelian sampling term was sampled from a normal distribution with mean zero and variance $(\sigma^2_{u_j}/2)(1\text{-}F)$ where F is the average inbreeding coefficient of the parents. The QTL genotype of an offspring was obtained by randomly sampling one allele from each parent.

4.2.3 Estimation of breeding values

The estimates of polygenic breeding values (EBV) for the production trait (on which artificial selection was based) were obtained from a BLUP animal model using PEST4 (Groeneveld and Kovac, 1990). The BLUP evaluation used the base population total additive variance $(\sigma^2_u + \sigma^2_a)$ and the phenotypic values uncorrected for the effects of the QTL. Thus, it was assumed that no information on the QTL was available to aid selection.

The main objective was to investigate the impact of selection based on BLUP EBV for the production trait on QTL allele frequencies. Selection schemes simulating this scenario were run for 40 generations. In addition, some schemes where run for 400 generations of selection on the production trait and in these cases simple phenotypic selection was used in order to reduce computation time. In these scenarios, selection was based on the phenotypic records for production, also uncorrected for the QTL effect.

4.2.4 Estimation of the genetic parameters

Restricted maximum likelihood using the (co)variance component estimation program VCE5 (Neumaier and Groeneveld, 1998) was used to estimate genetic variances and the genetic covariance and correlation between the traits. The phenotypic values for both traits were uncorrected for the effects of the QTL. The correlation obtained in this way would correspond to that estimated in practice when no prior knowledge of the QTL is available. To reduce computation time, the correlations were estimated every five generations. The pedigree file was truncated and only the offspring and parent generations at a particular time were considered to obtain the estimates. The estimate of genetic correlation ($\hat{\rho}$) was compared with the true additive polygenic correlation (ρ_a) and with the true total correlation (ρ_T). The true additive polygenic correlation was calculated as $\rho_a = \text{cov}(u_1, u_2) / \sigma_{u_1}\sigma_{u_2}$. The total correlation was calculated in a similar way but using the total genetic values (g_i) for both traits instead of the polygenic values.

4.2.5 Selection

The scenarios considered modelled artificial selection on the production trait and natural selection on the disease susceptibility trait. Each generation, the 30 males and the 30 females with the highest EBV for production were selected (i.e. standard truncation selection). Also, a culling strategy (modelling natural selection) based on the disease phenotype was employed, such that individuals whose phenotypic value for disease susceptibility exceeded a particular threshold (high positive value, since susceptible

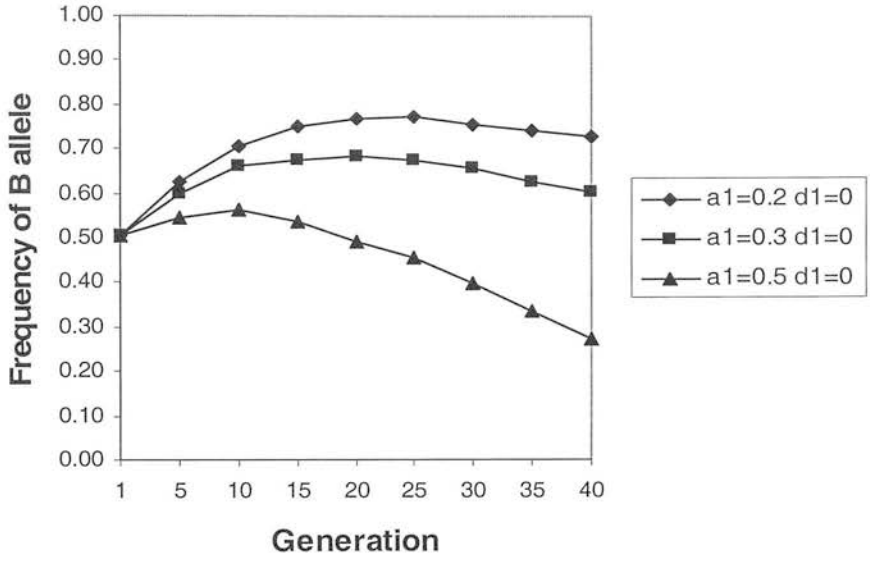
animals have, according to the definition of the disease model, high positive phenotypic values) were not allowed to be selected. Two different thresholds, corresponding to a disease incidence of 5% and 20% in the initial generation, were chosen. Thereafter the threshold values remained the same, however the disease incidence varied due to the combined effects of natural selection acting to remove affected individuals from the population and artificial selection on production affecting the allele frequency of the QTL.

4.3 Results

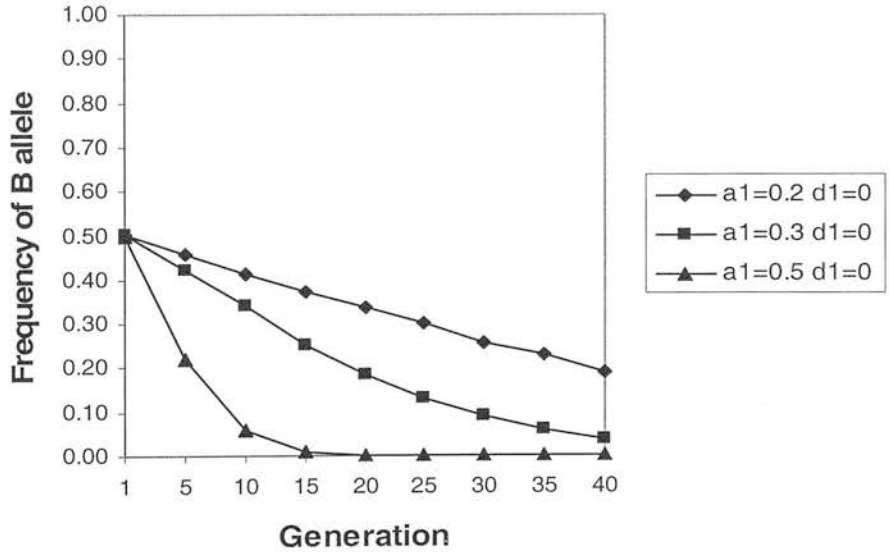
4.3.1 Additive QTL for the production trait

Figure 4.1 shows the change in frequency of the allele reducing disease susceptibility and production (i.e. frequency of the B allele, hereafter referred to as q) when the QTL had an additive effect for production (i.e., $d1 = 0$) and artificial selection was based on BLUP EBV. Different proportions of the variation for production accounted by the QTL and different initial disease incidences (i.e. the proportion of animals culled) in the population (20% or 5%) were considered. For both levels of initial disease incidence, q decreased over time, after an initial increase when the initial incidence was of 20%. The greater the proportion of variation the QTL accounted for in production ($a1 = 0.5$ versus $a1 = 0.3$ versus $a1 = 0.2$, where $a1$ is the additive QTL effect on trait 1) the faster q decreased and therefore p (the frequency of the A allele increasing disease susceptibility), increased.

Figure 4.1. Change in frequency of the allele reducing disease susceptibility (allele B) resulting from artificial selection on BLUP EBV for production when the QTL had an additive effect on production and the initial disease incidence in the population was 20% (a) or 5% (b).



a)



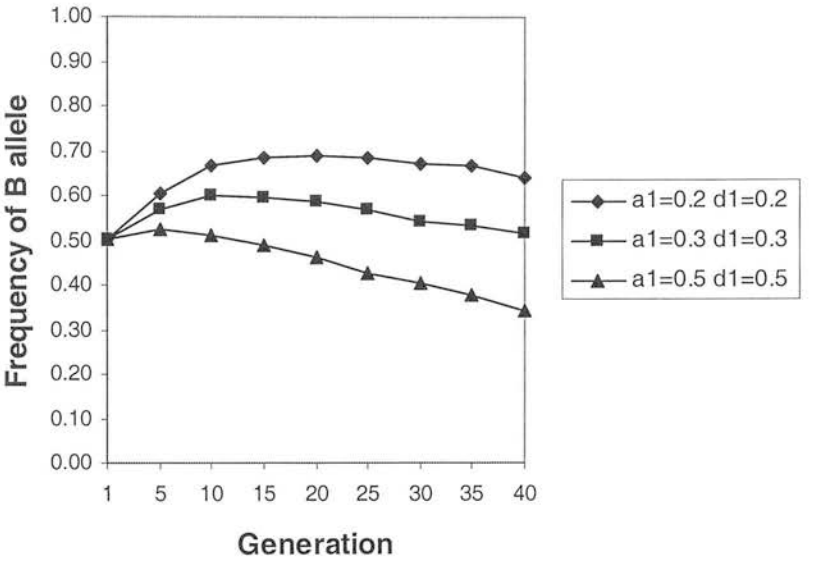
b)

The increase in q in the initial generations (first 10 to 20 generations, depending on the value of $a1$) when the initial incidence was 20% was due to the fact that animals with the favourable allele for production are culled as a result of natural selection (as this allele also increases disease susceptibility). The net effect was that more animals with the allele that reduced disease susceptibility were selected and q increased. After 10 to 20 generations, as the animals with high production became less susceptible to disease (i.e. as the genetic mean for disease susceptibility decreased due to natural selection, see Table 4.2), there was more scope for selection on production and therefore the frequency of the allele favourable for production (p) increased (and q decreased). After the initial increase in q, changes in frequency were small when the effect of the QTL was either $a1$ = 0.2 or 0.3 and, at generation 40, q was still at intermediate values (q = 0.73 for $a1$ = 0.2 and q = 0.6 for $a1$ = 0.3). When the disease incidence in the initial generation was 5%, the scope for selection on production was high from the start of the selection process and therefore q decreased across the whole selection period. The B allele was lost faster the greater the effect on production of the QTL (Figure 4.1b).

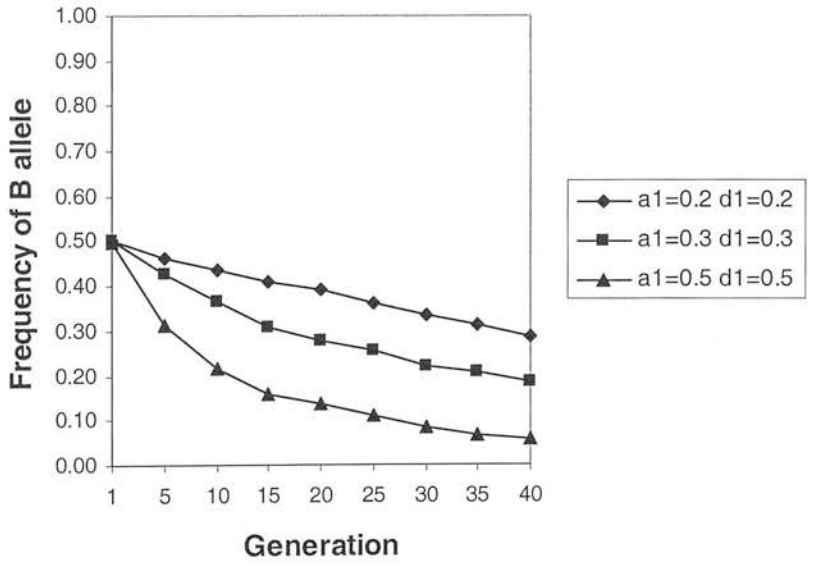
4.3.2 Dominant QTL for the production trait

Figure 4.2 shows the change in q when the mode of action of the QTL was dominant for production. The trends are similar to those observed when the QTL was additive but allele frequencies changed at a slower rate. For example, with $a1$ = 0.5, the allele reducing disease susceptibility was lost after 13 generations when the initial disease incidence was 5% and the QTL acted additively (Figure 4.1b) but was still segregating after 40 generations when the QTL was dominant (Figure 4.2b).

Figure 4.2. Change in frequency of the allele reducing disease susceptibility (allele B) resulting from artificial selection on BLUP EBV for production when the QTL had a dominant effect on production and the initial disease incidence in the population was 20% (a) or 5% (b).



a)



b)

As with the additive case, there was an initial increase in q when the initial disease incidence was high (20%) but this increase was less drastic when the QTL was dominant. Importantly, with $a1 = 0.2$ and a high disease incidence, after the initial increase, q stayed almost constant for many generations before it started to decrease slowly. Given these rates of change, 40 generations of selection were not enough to know if, in some scenarios, the frequency would stabilise at an intermediate value.

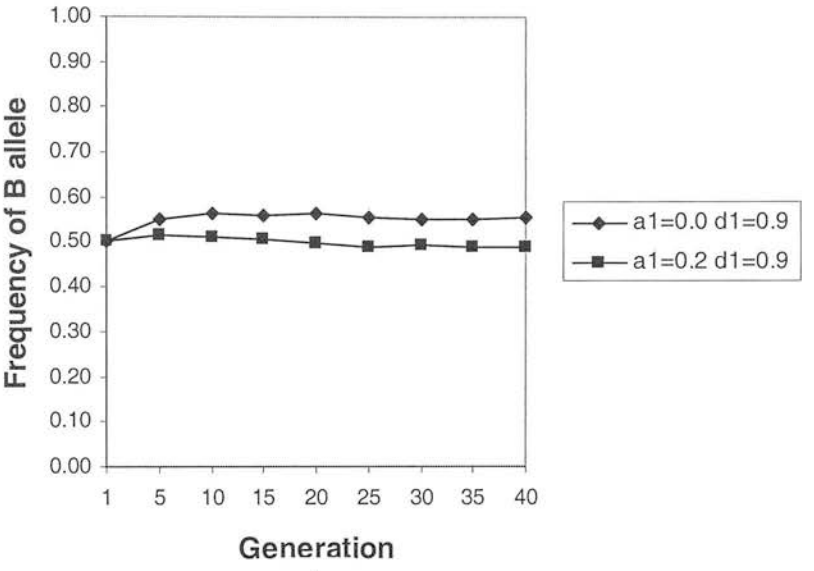
4.3.3 Overdominant QTL for the production trait

When the mode of action of the QTL production was overdominant, there was little change in gene frequency after the initial generations of selection (Figure 4.3a). In most overdominant scenarios investigated, the frequency of the allele approached equilibrium after as little as five generations. The initial disease incidence had relatively little effect on the equilibrium q value (Figure 4.3).

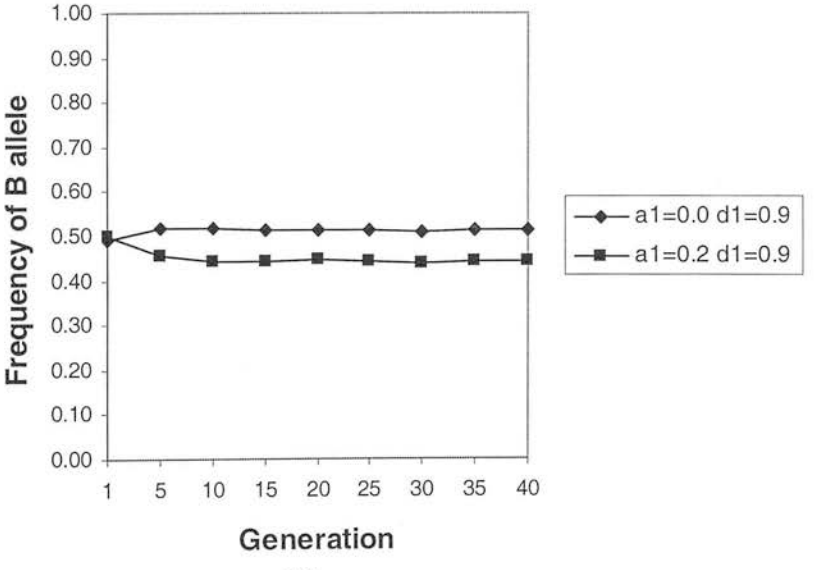
4.3.4 Selection for 400 Generations

In the scenarios where the QTL had an additive or dominant effect on the production trait the evolution of the QTL frequency after 40 generations of selection was unclear (Figures 4.1 and 4.2). In order to investigate the trend after generation 40, simulations were run applying artificial phenotypic selection on production for 400 generations assuming an initial disease incidence of 20%. Figure 4.4 shows that for the additive and dominant models, q continued to decline across generations and thus the expectation is that the allele reducing disease susceptibility will eventually be lost.

Figure 4.3. Change in frequency of the allele reducing disease susceptibility (allele B) resulting from artificial selection on BLUP EBV for production when the QTL had an overdominant effect on production and the initial disease incidence in the population was 20% (a) or 5% (b).



a)



b)

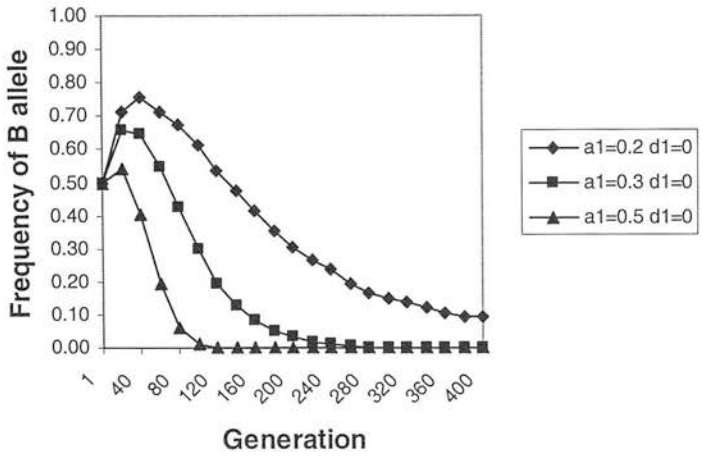
This allele was lost quicker when the mode of action of the QTL on production was additive compared to when it was dominant. Also, the greater the effect of the QTL on production, the faster the allele favourable for production was fixed. When the mode of action of the QTL on production was overdominant the frequency remained constant after reaching an equilibrium value in the initial generations.

4.3.5 Effect of different initial starting frequency

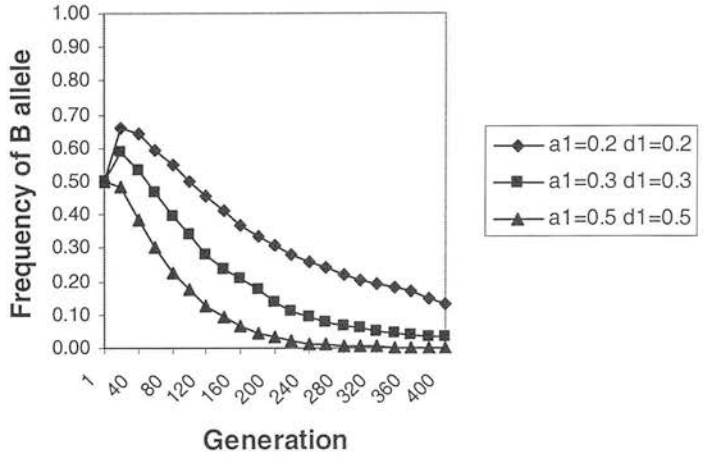
Figure 4.5 shows the changes in allele frequencies when the initial q was very high (and consequently p was very low), modelling a situation that might arise if B was the only QTL allele in the population and a new mutation (the A allele) favourable for production entered the population. The reverse situation; i.e., when the initial q was very low (0.05) was also considered, but results are only shown for the overdominant model, since A was quickly fixed in the population when the mode of action was additive or dominant.

When the mode of action was additive (Figure 4.5a) and the QTL effect on the production trait was either 0.3 or 0.5, q appeared to reach an equilibrium value around generation 200 or generation 100, respectively. This also occurred when $a1 = 0.0$ but only after 600 generations (results not shown). This equilibrium was however an artefact resulting from averaging across all replicates. Due to the high initial frequency, the allele was fixed in some (10 to 15) replicates within this period of time (Figure 4.6a). In comparison, when the starting frequency was 0.5 the allele was lost in all the replicates (Figure 4.6b).

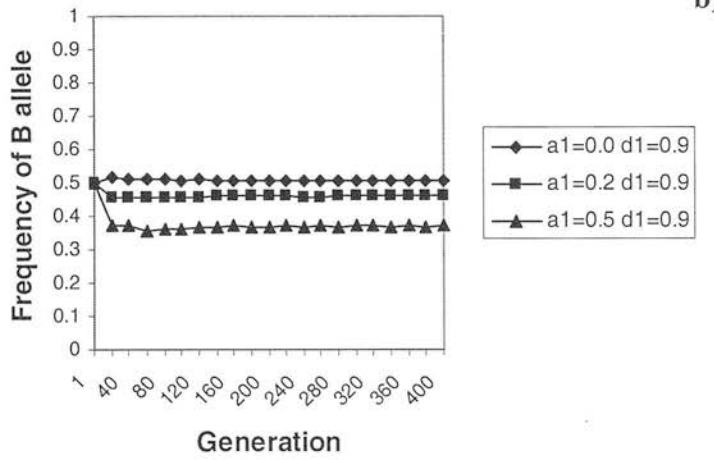
Figure 4.4. Change in frequency of the allele reducing disease susceptibility (allele B) resulting from artificial selection on phenotypes for production when the QTL had an additive (a), dominant (b), or overdominant effect (c) on production and selection was for 400 generations. The initial disease incidence was 20%.



a)



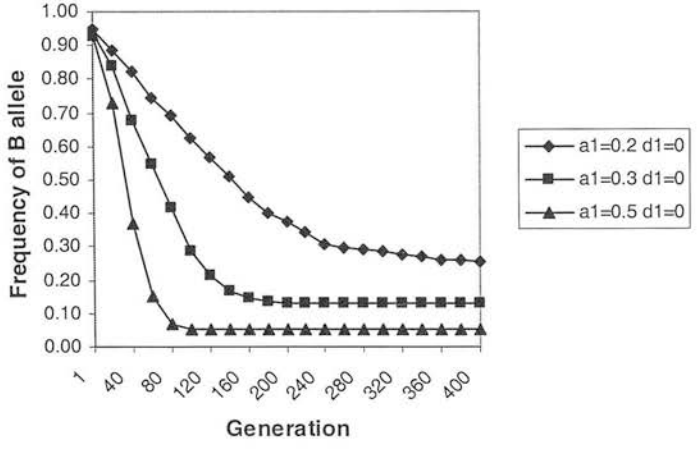
b)



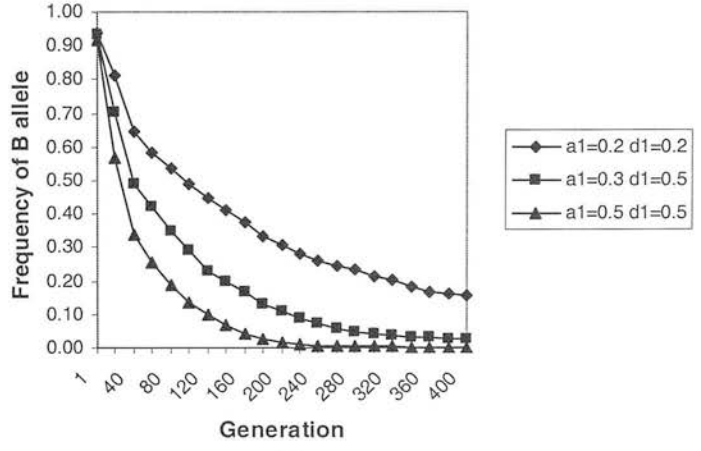
c)

When the mode of action was dominant, allele B was lost within 400 generations when $a1$ = 0.5 (Figure 4.5b). The allele was also lost when $a1$ = 0.2, and 0.3 but only after 400 generations (results not shown). When the mode of action was overdominant, the allele frequency reached equilibrium at an intermediate frequency, regardless of the starting frequency of the QTL or the $a1$ and $d1$ values (only $a1$=0.2 and $d1$=0.9 shown, Figure 4.5c).

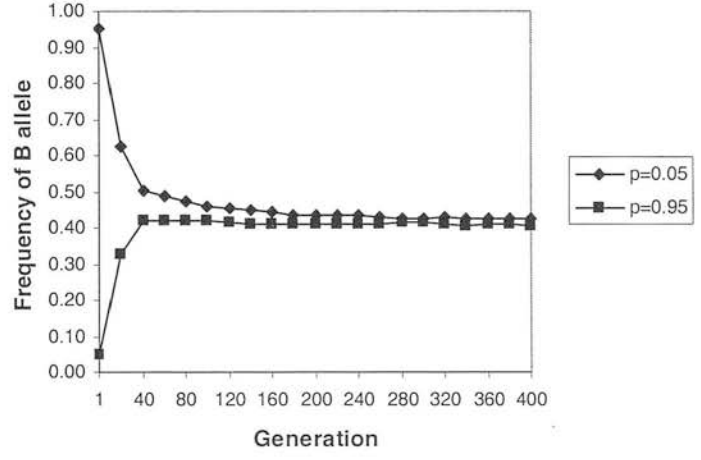
Figure 4.5. Effect of a different initial starting frequency of the allele favourable for reducing disease susceptibility (allele B) when the QTL had an additive (a) a dominant (b) or an overdominant (c) effect on production and the initial culling level in the population was 5%.



a)

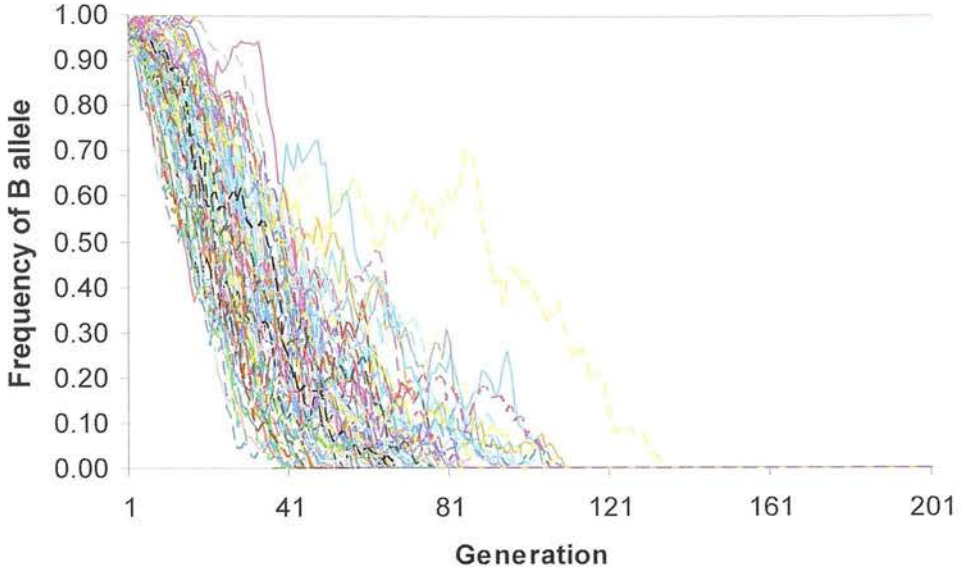


b)

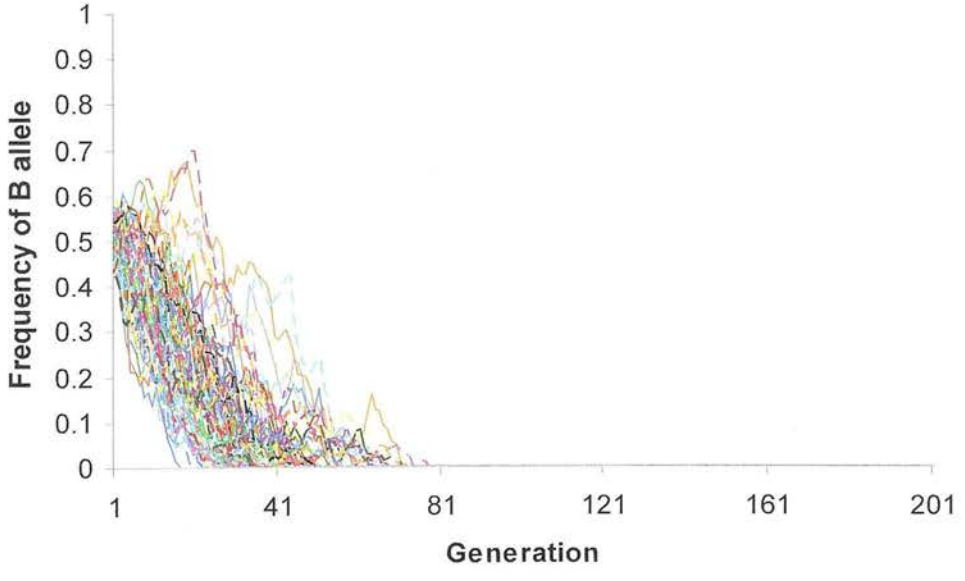


c)

Figure 4.6. Per replicate frequency of the allele favourable for reducing disease susceptibility (allele B) when the QTL had an additive effect on production ($a1 = 0.3$) and the starting frequency was 0.95 (a) or 0.5 (b).



a)



b)

4.3.6 Genetic Gain

Tables 4.2, 4.3 and 4.4 show the polygenic and total genetic mean (i.e., sum of the polygenic mean plus the QTL mean) over 40 generations for both traits when artificial selection was based on BLUP EBV for production. The results are shown for the scenarios $a1$ = 0.2 and $d1$ = 0.0 (additive), $a1$ = 0.2 and $d1$ = 0.2 (dominant) and $a1$ = 0.2 and $d1$ = 0.9 (overdominant). Results for corresponding models with different $a1$ and $d1$ values were similar. For each of the models, the polygenic and total mean for production was higher at each generation when the initial culling in the population was 5% versus 20%. The overdominant model had the lowest accumulated polygenic and total gains after 40 generations.

Artificial selection was on production only but there was a decrease in disease susceptibility as a result of natural selection imposed through the culling of affected animals. For all the models the polygenic and total means were lower (less negative) for disease susceptibility when the initial incidence was 5%, which means that, after 40 generations, a population with higher initial disease incidence will on average be healthier than a population with lower initial incidence. When the initial incidence in the population was 20% disease susceptibility was reduced most in the additive model, followed by the dominant model with the overdominant model having the lowest gain. However, this order was reversed when the initial disease incidence in the population was 5%. This can be explained by the changes in q during the 40 generations of selection on production. When the initial incidence was 20%, q was highest at generation 40 for the additive model compared to the other two models (Figures 4.1a,

4.2a and 4.3a), but q was lowest at generation 40 for the additive model when the initial incidence in the population is 5% (Figures 4.1b, 4.2b and 4.3b).

4.3.7 Disease Incidence

Tables 4.2, 4.3 and 4.4 also show the proportion of animals culled based on the disease phenotype (i.e., disease incidence). When the initial disease incidence was 20%, only around 7% of the population was culled by generation 40 under the additive and dominant models. Under the overdominant model the reduction in the total mean for disease susceptibility (–0.99 at generation 40) was less than under the additive and dominant models and therefore the disease incidence in the population was higher (10% at generation 40). When the initial incidence in the population was 5% the tendency was for the incidence to increase in the additive and dominant models, as a result of the allele reducing disease susceptibility going towards zero (Figures 4.1b and 4.2b). Under the overdominant model the disease incidence remained stable through generations.

Table 4.2. Polygenic (PG) and total (T) genetic means for production and disease susceptibility, and disease incidence (DI, proportion of animals culled based on phenotypes for the disease trait) across generations (Gen) when the QTL had an additive ($a1 = 0.2$; $d1 = 0$) effect on production, and the initial disease incidence was 20% or 5%.

	20% Culling					5% Culling				
	Production		Susceptibility			Production		Susceptibility		
Gen	PG	T	PG	T	DI	PG	T	PG	T	DI
0	0.00	0.00	0.00	-0.45	0.20	0.00	0.00	0.00	-0.45	0.05
1	0.32	0.30	-0.02	-0.56	0.18	0.40	0.40	-0.01	-0.43	0.05
2	0.61	0.57	-0.04	-0.65	0.14	0.73	0.74	-0.02	-0.41	0.05
3	0.91	0.86	-0.05	-0.72	0.12	1.05	1.07	-0.03	-0.38	0.06
4	1.21	1.15	-0.07	-0.76	0.11	1.37	1.39	-0.03	-0.37	0.05
5	1.52	1.45	-0.09	-0.80	0.10	1.68	1.71	-0.04	-0.34	0.06
10	2.73	2.64	-0.13	-0.89	0.08	2.93	2.97	-0.08	-0.29	0.06
20	5.66	5.55	-0.25	-1.04	0.06	5.86	5.93	-0.19	-0.23	0.06
30	8.44	8.34	-0.33	-1.10	0.06	8.61	8.71	-0.28	-0.09	0.07
40	11.02	10.93	-0.40	-1.14	0.06	11.19	11.32	-0.37	-0.01	0.07

Table 4.3. Polygenic (PG) and total (T) genetic means for production and disease susceptibility, and disease incidence (DI, proportion of animals culled based on phenotypes for the disease trait) across generations (Gen) when the QTL had a dominant ($a1$=0.2; $d1$=0.2) effect on production, and the initial disease incidence was 20% or 5%.

	20% Culling					5% Culling				
	Production		Susceptibility			Production		Susceptibility		
Gen	PG	T	PG	T	DI	PG	T	PG	T	DI
0	0.00	0.10	0.00	-0.45	0.20	0.00	0.10	0.00	-0.45	0.05
1	0.32	0.40	-0.02	-0.56	0.17	0.39	0.50	-0.01	-0.42	0.05
2	0.61	0.67	-0.04	-0.63	0.15	0.72	0.84	-0.02	-0.40	0.05
3	0.90	0.95	-0.06	-0.69	0.12	1.04	1.15	-0.03	-0.39	0.05
4	1.20	1.24	-0.07	-0.73	0.11	1.37	1.48	-0.04	-0.39	0.06
5	1.50	1.53	-0.09	-0.75	0.11	1.68	1.80	-0.05	-0.39	0.05
10	2.68	2.69	-0.14	-0.85	0.09	2.92	3.05	-0.08	-0.37	0.06
20	5.54	5.55	-0.25	-0.97	0.08	5.87	6.01	-0.19	-0.39	0.06
30	8.23	8.25	-0.34	-1.01	0.08	8.65	8.81	-0.27	-0.32	0.06
40	10.76	10.80	-0.42	-1.06	0.08	11.26	11.42	-0.33	-0.26	0.05

Table 4.4. Polygenic (PG) and total (T) genetic means for production and disease susceptibility, and disease incidence (DI, proportion of animals culled based on phenotypes for the disease trait) across generations (Gen) when the QTL had an overdominant ($a1 = 0.2$; $d1 = 0.2$) effect on production, and the initial disease incidence was 20% or 5%.

	20% Culling					5% Culling				
	Production		Susceptibility			Production		Susceptibility		
Gen	PG	T	PG	T	DI	PG	T	PG	T	DI
0	0.00	0.45	0.00	-0.45	0.20	0.00	0.45	0.00	-0.45	0.05
1	0.29	0.73	-0.02	-0.53	0.17	0.36	0.82	-0.01	-0.42	0.05
2	0.56	0.98	-0.04	-0.58	0.15	0.68	1.12	-0.02	-0.41	0.05
3	0.83	1.24	-0.06	-0.62	0.15	0.99	1.45	-0.03	-0.41	0.05
4	1.10	1.51	-0.08	-0.65	0.14	1.29	1.74	-0.04	-0.41	0.05
5	1.37	1.77	-0.09	-0.66	0.13	1.58	2.04	-0.05	-0.43	0.05
10	2.42	2.83	-0.16	-0.73	0.12	2.77	3.23	-0.08	-0.42	0.05
20	4.93	5.35	-0.30	-0.83	0.12	5.55	6.02	-0.15	-0.50	0.05
30	7.33	7.76	-0.40	-0.90	0.10	8.16	8.62	-0.20	-0.53	0.04
40	9.63	10.06	-0.50	-0.99	0.10	10.61	11.08	-0.24	-0.54	0.04

4.3.8 Genetic Correlations

Table 4.5 shows the true additive polygenic (ρ_a), true total (ρ_T) and estimated ($\hat{\rho}$) genetic correlations between the two traits. For clarity purposes, one example of each of the additive, dominant and overdominant models are shown; however similar trends were obtained for the other scenarios investigated.

Table 4.5. Effect of the mode of action of the QTL for production on the true polygenic (ρ_a), true total (ρ_T) and estimated ($\hat{\rho}$) genetic correlations between production and disease susceptibility over generations (Gen).

	Additive $a1 = 0.5, d1 = 0.0$			**Dominant** $a1 = 0.5, d1 = 0.5$			**Overdominant** $a1 = 0.2, d1 = 0.9$		
Gen	ρ_a	ρ_T	$\hat{\rho}$	ρ_a	ρ_T	$\hat{\rho}$	ρ_a	ρ_T	$\hat{\rho}$
1	0.02	0.36	0.47	0.00	0.19	0.48	0.01	-0.16	0.21
5	0.02	0.31	0.30	-0.01	0.13	0.28	0.00	-0.18	0.08
10	0.02	0.31	0.26	0.00	0.13	0.30	0.01	-0.20	0.15
15	0.02	0.33	0.30	0.01	0.13	0.32	0.00	-0.22	0.05
20	0.02	0.34	0.36	0.00	0.13	0.27	0.00	-0.23	0.16
25	0.01	0.35	0.34	0.00	0.14	0.31	-0.03	-0.24	0.15
30	0.00	0.37	0.39	-0.01	0.14	0.32	-0.03	-0.27	0.09
35	0.00	0.36	0.36	-0.01	0.14	0.35	0.01	-0.27	0.08
40	0.02	0.32	0.32	0.00	0.12	0.25	0.01	-0.29	0.18

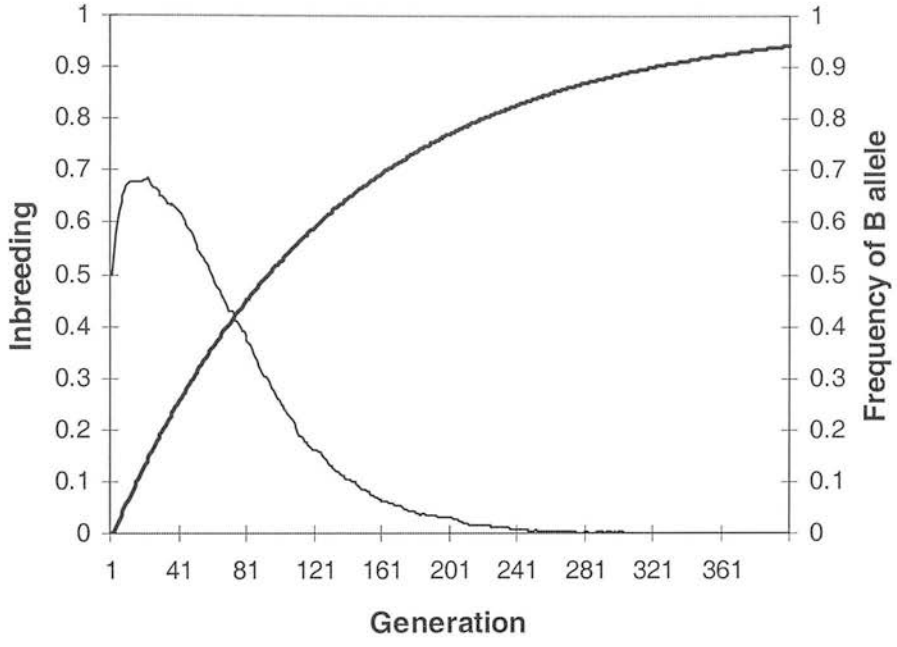
As expected, in the initial population ρ_a was not significantly different from zero and remained at this value throughout each generation of selection. The estimated genetic correlation ($\hat{\rho}$) differed from ρ_a as the two traits were truly correlated due to the QTL and the estimation method used phenotypes uncorrected for the QTL effect. When the

QTL had an additive mode of action on production $\hat{\rho}$ and ρ_T were of the same sign and of similar magnitude. This would suggest that when the information on the QTL is ignored, $\hat{\rho}$ is a good estimate of the actual underlying total genetic correlation. When the mode of action of the QTL on production was dominant $\hat{\rho}$ was of the same sign but clearly overestimated ρ_T. Lastly, when the mode of action was overdominant $\hat{\rho}$ and ρ_T were very different and even had opposite signs. This indicates that in certain situations the estimated correlation between two traits that are affected by a single QTL can be very inaccurate. The inaccurate estimates may even suggest that the two traits are controlled independently when they are not. For instance, when the mode of action of the QTL on production was overdominant and $a1 = 0$, the estimated genetic correlation was not significantly different from zero (results not shown) indicating that the two traits are controlled independently, despite the fact that the pleiotropic QTL was segregating in the population.

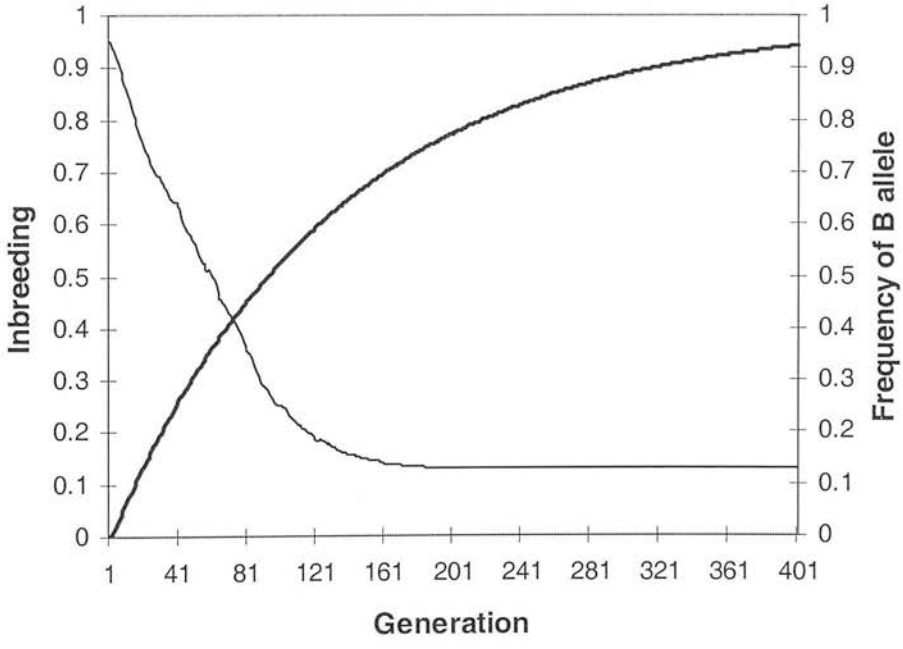
4.3.9 Inbreeding

Figure 4.7 shows the accumulation of inbreeding in the population when assuming an additive QTL model for production. Similar results were found for the dominant and overdominant models. The inbreeding was similar regardless of the starting frequency and was approximately 0.25 after 40 generations. The mean inbreeding reached almost 0.95 after 400 generations of selection.

Figure 4.7. Mean inbreeding (thick line) and frequency of the allele favourable for reducing disease susceptibility (thin line) across generations when the QTL had an additive effect on production ($a1$ = 0.3) and the starting frequency was 0.95 (a) or 0.5 (b).



a)



b)

4.4 Discussion

This study has investigated the evolution of allele frequencies for a QTL with pleiotropic effect on production and disease susceptibility when artificial selection is applied to increase production. Navarro (2006a,b) suggested that two traits, that seemed to be uncorrelated, may be controlled by a pleiotropic QTL (dominant for disease susceptibility and overdominant for production) and as a result the disease susceptibility allele could be segregating at intermediate frequencies in a commercial population under artificial selection for production. Here, we show that it is possible to find such QTL segregating in populations undergoing selection for a variety of situations. The changes in frequency of the QTL over time clearly depend on the mode of action of the QTL on production; however, regardless of this, we showed that the QTL could still be segregating in the population after 40 generations of selection. In fact, under certain scenarios, the QTL alleles remained at intermediate frequencies across generations.

In the case of the additive and dominant models, the QTL favourable for production was eventually fixed ($p = 1$) but this did not happen for several hundreds of generations. This is highly relevant for practical breeding programmes as, even with a generation interval as short as one year, fixation will not occur for a long time highlighting the importance of detecting pleiotropic QTL and using molecular information to manipulate their allele frequencies. When the QTL for production was overdominant an intermediate equilibrium frequency was reached and the QTL remained at this frequency throughout generations.

Although for the additive and dominant models the QTL was still segregating after many generations, it is important to note that the allele that reduces disease susceptibility will eventually be lost (Figure 4.4) under most scenarios. Also, under the additive model, when the initial frequency of the allele that increases disease resistance was very high, some populations became fixed for this allele (Figure 4.6a). This does not happen for either the dominant or overdominant models.

In this study the amount of variation accounted for by the QTL for disease susceptibility was assumed to be large (85%). In scenarios where this allele is lost, this would happen more quickly when the QTL has a smaller effect on the trait. As expected, the changes in the QTL frequency depend on the amount of variation the QTL accounts for both traits. Under the additive and dominant models for production, the greater the proportion of variation explained by the QTL for production the faster the allele favourable for production was fixed, and the allele reducing disease susceptibility was lost. Under these models, when the initial disease incidence in the population was 20%, there was an initial decrease in the disease susceptibility allele as the high production animals, with AA genotype, were culled. However, as the high production animals were improved genetically for disease susceptibility (via the background polygenes) more of these animals with the favourable QTL allele for production were selected and the frequency of the A allele increased.

Accurate estimation of the genetic correlations among traits in the selection objective is an integral part of any successful breeding programme. Such correlations will help to

determine whether or not we can expect correlated responses when selecting for a particular trait or for a combination of traits. Here we simulated a polygenic genetic correlation of zero between the two traits. When the pleiotropic QTL was segregating the two traits will be correlated to some degree through the QTL. Here we show that the mode of action of the QTL on production had an effect on the estimated genetic correlation and that under some circumstances this correlation can be very different from the true (simulated) correlation. Where the QTL had an additive effect on production, the estimated correlation was a good indication of the total genetic correlation. However, under the non-additive models the estimated correlation was far from the true value and in the case of the overdominant model the estimated and true correlations had even opposite signs. This has important implications in terms of selection in a breeding programme as, traditionally, a single genetic correlation assumed to be caused by additive polygenes is estimated in the absence of information on QTL. When the phenotypic values were corrected for the QTL, estimated genetic correlations did not differ significantly from zero (results not shown). This is expected as the polygenic genetic correlation simulated was zero and the effects of the QTL have been accounted for, thereby removing the correlation that had been generated by the QTL.

Originally QTL mapping experiments have focused on single traits. However, the ability to simultaneously record many traits and the advances in statistical methodology to detect QTL has led to attempts in detecting pleiotropic QTL (e.g. Freyer et al., 2003; Varona et al., 2004). This information is very important in the context of designing a breeding programme aimed to improve several traits simultaneously such as production

and disease type traits. Examples of pleiotropic QTL found in livestock species include QTL for milk component traits in dairy cattle (e.g. Feyer et. al., 2003; Schrooten and Bovenhuis, 2004), and lean meat and susceptibility to stress in pigs (Nicholas, 1996). Here, we utilise estimates of QTL effects that were generated from a study of ascites in chickens (Navarro, 2006b). Nevertheless, the scenarios could be typical of any livestock species where a trait like production has been artificially selected for, for many generations.

4.5 Conclusions

The scenario simulated (selection for production when no information is known about a pleiotropic QTL that affects both production and disease susceptibility) would be representative of animal breeding schemes over the last number of decades. Results show that a QTL allele unfavourable for the trait under artificial selection can remain segregating in a population for many generations. The length of time it remains in the population depends on the mode of action of the pleiotropic QTL on both traits. Assuming that the QTL is dominant for disease susceptibility, the allele will eventually be lost if the mode of action is additive or dominant for production, but it will reach an equilibrium frequency if the mode of action is overdominant. Another important finding is that when a pleiotropic QTL is segregating, estimates of the genetic correlation between the two traits may be misleading having the potential to cause unwanted effects if these estimates were used in breeding programmes. The findings of this study have important implications for practical breeding programs. For example, if a pleiotropic

QTL existed that favoured the heterozygotes for a production trait (i.e. overdominant) then it would be very difficult to remove the disease susceptibility allele via traditional selection methods. In such situations the use of QTL information could be of great benefit.

CHAPTER FIVE

BENEFITS OF USING AN IDENTIFIED PLEIOTROPIC QTL WITH ANTAGONISTIC EFFECTS ON TWO TRAITS

5.1 Introduction

Many studies have looked at the potential benefits of using gene or marker information to aid selection in animal breeding programmes (e.g. Kashi et al., 1990; Gibson 1994; Ruane and Colleau, 1995; Villanueva et al., 2004). In most cases marker (MAS) or gene assisted selection (GAS) increased genetic gain, especially for sex-limited traits, traits of low heritability or traits for which records were not available before selection (Meuwissen and Goddard, 1996). In general, these studies focused on single trait selection when the QTL had an additive effect on the trait of interest. Researchers have started to look for pleiotropic QTL as these can have important implications in MAS. For example, if a QTL that increases production has been detected, MAS should offer benefits for improving this trait, at least in the early generations. However, if the underlying QTL unknowingly affects other important traits MAS could lead to unexpected and suboptimal responses. Therefore, it is important to assess the impact of using a QTL that has an effect on two traits as the expectations may be different to those from the single trait scenario.

There are a number of pleiotropic QTL being detected in livestock species. In dairy cattle, QTL affecting more than one milk production trait have been found (e.g. Feyer et al., 2003; Grisart et al., 2002). Schrooten et al. (2004) detected 59 chromosomal regions throughout the genome affecting various combinations of traits. In beef cattle the myostatin gene is associated with increased lean meat yield but also with increased calving difficulty. In pigs the halothane gene is responsible for an increase in lean meat yield, but can also result in the low quality meat or susceptibility to stress. In chickens, Navarro (2006a,b) found evidence of a

pleiotropic QTL that had an effect on weight and ascites-related traits in chickens, despite an estimated polygenic correlation not significantly different from zero. In this latter study, it was determined that the mode of action of the putative QTL was likely to be overdominant for production, and dominant for ascites resistance. It was hypothised that this particular genetic model together with selection on production was a potential cause for the continual segregation of the allele conferring disease susceptibility. In chapter 4 we showed that such a QTL remained segregating in the population, despite being under continual selection for production for many generations. In such situations it would be necessary to use genetic markers to control or eliminate the disease susceptibility allele.

The objective of this chapter was to investigate genetic gains and changes in allele frequencies when a QTL affecting production and disease susceptibility was used in selection. GAS schemes were compared with those ignoring QTL information. We looked at several selection criteria and at several modes of action of the QTL on production when the QTL was dominant for disease susceptibility.

5.2 Materials and Methods

5.2.1 Genetic model

The two traits were assumed to be controlled by additive polygenes plus a biallelic pleiotropic QTL (alleles A and B). The total genetic value of an individual i for trait j, was $g_{ij}= v_{ij} + u_{ij}$, where v_i is the genotypic value due to the QTL, and u_i is the

polygenic effect. The polygenic heritabilities (h^2) were 0.5 and 0.1 for traits one and two, respectively. These values are representative of the heritabilites for production-type traits (0.5) and disease-type traits (0.1). The polygenic correlation between the two traits was zero. The genotypic value due to the QTL was a_j, d_j or $-a_j$, where a_j is the additive effect on trait j, defined as the half the difference between the two homozygotes, and dj is the dominance effect on trait j, defined as the difference between the heterozygote and the average of the two homozygotes (Falconer and Mackay, 1996). The additive variance explained by the QTL in the base generation for trait j was $\sigma^2_{aj} = 2pq\alpha_j^2$ where p is the initial frequency of the A allele, $q = (1\text{-}p)$ and $\alpha_j = [a_j + d_j\ (q - p)]$ (Falconer and Mackay, 1996). The dominance variance was $\sigma^2_{dj} = (2pqd_j)^2$. The initial p was 0.5.

Different genetic models were considered by changing the values of a_1 and d_1 (i.e. values for the production trait). The values of a and d for disease susceptibility (i.e. a_2 and d_2) were kept constant. The proportion of the total genetic variation explained by the QTL for both traits is shown in Table 5.1. The genetic model assumed that the allele A increased production and susceptibility to disease.

Table 5.1. Summary of the additive (a) and dominance values (d), corresponding genotypic values and percentage of the total variation (% Gen Var) accounted for by the QTL for production and disease susceptibility.

	Genotypic Values			
Model	**A[a]A**	**AB[b]**	**BB**	**% Gen Var**
Production				
$a_1 = 0.2, d_1 = 0.0$	0.2	0.0	-0.2	4%
$a_1 = 0.2, d_1 = 0.2$	0.2	0.2	-0.2	6%
$a_1 = 0.2, d_1 = 0.9$	0.2	0.9	-0.2	31%
Disease Susceptibility $a_2 = 0.9, d_2 = 0.9$	0.9	-0.9	-0.9	85%

[a] Allele 'A' increases production and disease susceptibility
[b] Allele 'B' decreases production and disease susceptibility

5.2.2 Simulation of the population

One-hundred and twenty individuals (60 males and 60 females) were simulated in the base generation (t = 0). Generation 1 (t = 1) was obtained from the matings of individuals selected at t = 0. The phenotypic value for an individual (i) was obtained by adding the total genetic value to an environmental component. The polygenic value and environmental components were obtained from normal distributions with mean zero and variances σ^2_{uj} and σ^2_{ej}, respectively. The polygenic and environmental variances summed to one for each trait.

For generations $t > 0$ the polygenic effect of the offspring was obtained by adding a random Mendelian sampling term to the average of the polygenic effects of their parents. The Mendelian sampling term was sampled from a normal distribution with mean zero and variance $(\sigma^2_{uj}/2)(1 - F)$ where F is the average inbreeding coefficient

of the parents. The QTL genotype of an offspring was obtained by randomly sampling one allele from each parent.

5.2.3 Estimation of breeding values

In the schemes where the QTL information was used, the effect of the QTL on both traits, and the QTL genotype for each individual were assumed to be known without error. The total estimated breeding value for an individual i for trait j, was calculated as $TEBV_{ij} = EBVu_{ij} + BV_{QTL_{ij}}$, where $EBVu_{ij}$ is the estimate of the polygenic breeding value for trait j, and $BV_{QTL_{ij}}$ is the known breeding value due to the QTL for trait j. The breeding values due to the QTL were calculated as $2q\alpha_j$, $(q - p)\alpha_j$, and $-2p\alpha_j$ for genotypes AA, AB, and BB, respectively (Falconer and Mackay, 1996). In these schemes, the base population polygenic variances (σ^2_{u1} and σ^2_{u2}) were used in the BLUP evaluation and the phenotypic values were corrected for the effects of the QTL. A bivariate BLUP animal model was used to estimate the polygenic breeding values using PEST4 (Groeneveld and Kovac, 1990).

In the schemes where the QTL information was ignored, the total estimated breeding values for both traits were those obtained from a bivariate BLUP animal model using the base population total additive variances ($\sigma^2_{u1} + \sigma^2_{a1}$ and $\sigma^2_{u2} + \sigma^2_{a2}$) and the phenotypic values uncorrected for the effects of the QTL.

5.2.4 Selection

Several scenarios including artificial selection on different selection indices were considered. To construct these indices, different weights on production (trait 1) and disease susceptibility (trait 2) were considered:

a) $I_1 = TEBV_1$ (i.e., selection on the production trait only)

b) $I_2 = 5TEBV_1 - TEBV_2$ (i.e., selection on both the production trait and the disease susceptibility trait, but more emphasis on production)

c) $I_3 = TEBV_1 - TEBV_2$ (i.e., selection on both the production trait and the disease susceptibility trait with equal emphasis on both traits)

For each index, schemes using the QTL information were compared to those ignoring the QTL.

Each generation, the 30 males and the 30 females with the highest index were selected (i.e. standard truncation artificial selection). In addition, a culling strategy based on the disease phenotype was employed modelling natural selection. Individuals whose phenotypic value for disease susceptibility exceeded a particular positive threshold (according to the definition of the disease model, susceptible animals have high positive phenotypic values) were not allowed to be selected. The threshold was obtained by setting the disease incidence in the initial generation at 20%. Thereafter the threshold value remained constant, however the disease incidence varied due to the combined effects of natural selection acting to remove affected individuals from the population and artificial selection on the index.

In practice, one may consider selection schemes that reduce disease susceptibility quickly. A simple example of such a scheme would involve culling all AA animals before selecting on the index. In order to quantify the loss in response to selection in the production trait as a result of this, the above selection schemes were re-run, but with all AA animals culled before selecting on the index.

5.3 Results

For clarity of presentation only results for indexes I_1 and I_3 three are presented. The results for I_2 are intermediate between those for I_1 and I_3.

5.3.1 Selection on production only

Table 5.2 shows the comparison of polygenic and total genetic gain (sum of the polygenic and QTL gain) when using and ignoring the QTL in selection when the effect of the QTL on production was completely additive and selection was on index I_1 (i.e., selection on the production trait only). It also shows the disease incidence measured as the proportion of animals culled on phenotypes for the disease trait. The polygenic and total gain for production were similar in both schemes for the first few generations but in later generations both gains were greater when ignoring than when using the QTL. By generation 40, the scheme ignoring the QTL yielded approximately 5% more total gain than the scheme using the QTL. Despite the fact that artificial selection was on production only, there was a response in disease susceptibility as animals exceeding the disease threshold were culled from the population. Slightly more polygenic gain (i.e., gain is more negative) was achieved in the scheme using the QTL but the total gain was less. The frequency of the allele

reducing disease susceptibility is shown in Figure 5.1a. When using the QTL, the frequency the allele that reduces disease susceptibility (and production) rose to a peak of 0.67 at generation 10 and was back to 0.49 by generation 40. When ignoring the QTL the frequency increased to a peak of 0.78 at generation 20 and was at 0.73 by generation 40. These frequency changes are reflected in changes in disease incidence in the population. When using the QTL the disease incidence was higher (Table 5.2) as the frequency of the disease susceptibility allele was higher, and therefore more animals were culled.

Table 5.3 shows the comparison of both schemes (using and ignoring the QTL) when the effect of the QTL on production was dominant. The trend was very similar to when the effect of the QTL was additive, with the polygenic and total gain being approximately 4% lower by generation 40 for the schemes using QTL information. The trend observed for the genetic mean for disease susceptibility was also similar to the additive model, with slightly more polygenic gain and lower overall genetic gain. The frequency of the QTL reached a higher peak (0.69) and was higher at generation 40 (0.63) when the QTL was ignored compared to when the QTL was used (Figure 5.1b), resulting in a lower disease incidence in the population (Table 5.3). Compared to the additive model, the frequency of the allele reducing disease susceptibility was lower for the dominant model across generations and therefore the disease incidence was slightly higher for the dominant model.

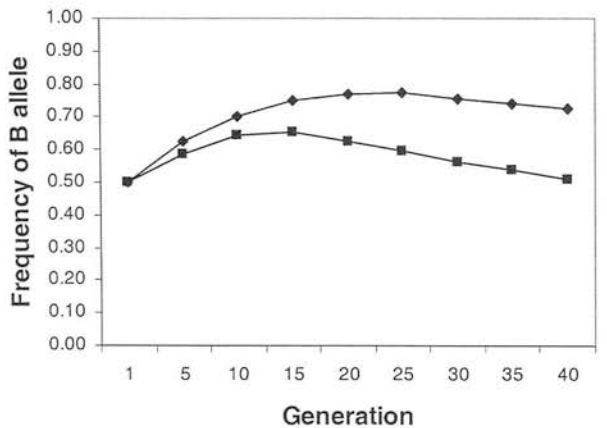
When the effect of the QTL on production was overdominant about 4% additional polygenic and total genetic gain in production was achieved at generation 40 in the schemes using the QTL (Table 5.4). Polygenic gain was higher for disease susceptibility when using the QTL and total genetic gain was similar. The frequency of the QTL that reduces disease susceptibility peaked at slightly higher values when using the QTL information but the frequency at generation 40 was similar (0.50) for both schemes (Figure 5.1c). Disease incidence throughout selection did not vary much between schemes (Table 5.4).

Table 5.2. Polygenic (PG) and total (T) genetic means for production and disease susceptibility, index response (RI), and disease incidence (DI) across generations (Gen) using or ignoring the QTL when selection was on production only (I_1) and the effect of the QTL on production was additive ($a_1 = 0.2$; $d_1 = 0$).

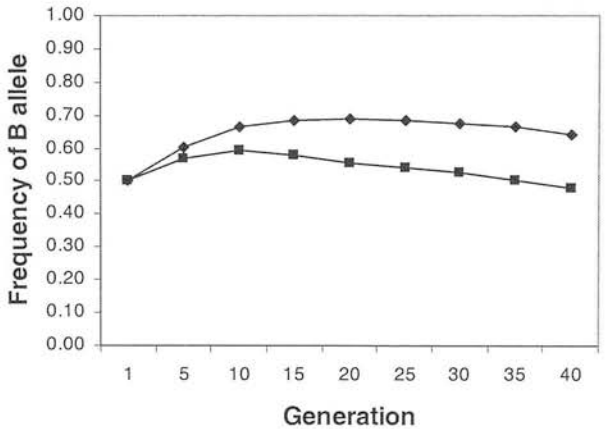
	Using QTL information					Ignoring QTL information					
	Production		Susceptibility			Production		Susceptibility			
Gen	PG	T	PG	T	DI	PG	T	PG	T	DI	RI[1]
0	0.00	0.00	0.00	-0.45	0.20	0.00	0.00	0.00	-0.45	0.20	0.00
1	0.32	0.31	-0.02	-0.52	0.17	0.32	0.30	-0.02	-0.56	0.18	0.01
2	0.61	0.58	-0.05	-0.61	0.14	0.61	0.57	-0.04	-0.65	0.14	0.01
3	0.90	0.87	-0.06	-0.65	0.14	0.91	0.86	-0.05	-0.72	0.12	0.01
4	1.19	1.15	-0.08	-0.70	0.12	1.21	1.15	-0.07	-0.76	0.11	0.00
5	1.48	1.43	-0.09	-0.73	0.12	1.52	1.45	-0.09	-0.80	0.10	-0.02
10	2.66	2.59	-0.14	-0.84	0.11	2.73	2.64	-0.13	-0.89	0.08	-0.05
20	5.45	5.41	-0.29	-0.90	0.11	5.66	5.55	-0.25	-1.04	0.06	-0.14
30	8.05	8.03	-0.38	-0.90	0.11	8.44	8.34	-0.33	-1.10	0.06	-0.31
40	10.47	10.48	-0.48	-0.87	0.10	11.02	10.93	-0.40	-1.14	0.06	-0.45

[1] RI= Total gain using QTL – Total gain ignoring QTL

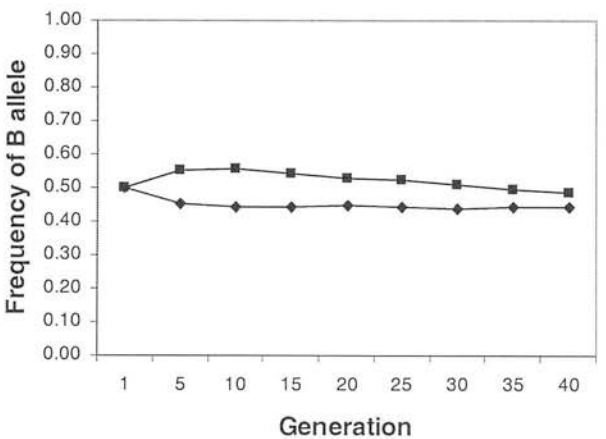
Figure 5.1. Frequency of the allele reducing disease susceptibility (allele B) across generations, using (■) or ignoring (♦) the QTL when selection was on production only (I_1) and the effect of the QTL on production was additive (a), dominant (b) or overdominant (c).



a)



b)



c)

Table 5.3. Polygenic (PG) and total (T) genetic means for production and disease susceptibility, index response (RI), and disease incidence (DI) across generations (Gen) using or ignoring the QTL when selection was on production only (I_1) and the effect of the QTL on production was dominant ($a_1 = 0.2$; $d_1 = 0.2$).

	Using QTL information					Ignoring QTL information					
	Production		Susceptibility			Production		Susceptibility			
Gen	PG	T	PG	T	DI	PG	T	PG	T	DI	RI[1]
0	0.00	0.10	0.00	-0.45	0.20	0.00	0.10	0.00	-0.45	0.20	0.00
1	0.32	0.41	-0.02	-0.53	0.17	0.32	0.40	-0.02	-0.56	0.17	0.01
2	0.60	0.67	-0.04	-0.59	0.15	0.61	0.67	-0.04	-0.63	0.15	0.00
3	0.89	0.96	-0.07	-0.63	0.15	0.90	0.95	-0.06	-0.69	0.12	0.01
4	1.18	1.24	-0.08	-0.67	0.13	1.20	1.24	-0.07	-0.73	0.11	0.00
5	1.46	1.52	-0.10	-0.69	0.13	1.50	1.53	-0.09	-0.75	0.11	-0.01
10	2.59	2.65	-0.16	-0.76	0.12	2.68	2.69	-0.14	-0.85	0.09	-0.04
20	5.33	5.40	-0.32	-0.84	0.12	5.54	5.55	-0.25	-0.97	0.08	-0.15
30	7.87	7.96	-0.46	-0.92	0.11	8.23	8.25	-0.34	-1.01	0.08	-0.29
40	10.29	10.40	-0.56	-0.92	0.11	10.76	10.80	-0.42	-1.06	0.08	-0.40

[1] RI= Total gain using QTL – Total gain ignoring QTL

Table 5.4. Polygenic (PG) and total (T) genetic means for production and disease susceptibility, index response (RI), and disease incidence (DI) across generations (Gen) using or ignoring the QTL when selection was on production only (I_1) and the effect of the QTL on production was overdominant ($a_1 = 0.2$; $d_1 = 0.9$).

	Using QTL information					Ignoring QTL information					
	Production		Susceptibility			Production		Susceptibility			
Gen	PG	T	PG	T	DI	PG	T	PG	T	DI	RI[1]
0	0.00	0.45	0.00	-0.45	0.20	0.00	0.45	0.00	-0.45	0.20	0.00
1	0.32	0.75	-0.02	-0.53	0.17	0.29	0.73	-0.02	-0.53	0.17	0.02
2	0.60	1.02	-0.05	-0.59	0.15	0.56	0.98	-0.04	-0.58	0.15	0.04
3	0.87	1.29	-0.07	-0.61	0.15	0.83	1.24	-0.06	-0.62	0.15	0.05
4	1.15	1.57	-0.08	-0.64	0.14	1.10	1.51	-0.08	-0.65	0.14	0.06
5	1.43	1.84	-0.10	-0.64	0.14	1.37	1.77	-0.09	-0.66	0.13	0.07
10	2.50	2.93	-0.16	-0.71	0.13	2.42	2.83	-0.16	-0.73	0.12	0.10
20	5.16	5.58	-0.33	-0.81	0.12	4.93	5.35	-0.30	-0.83	0.12	0.23
30	7.71	8.17	-0.46	-0.91	0.11	7.33	7.76	-0.40	-0.90	0.10	0.41
40	10.11	10.57	-0.56	-0.99	0.10	9.63	10.06	-0.50	-0.99	0.10	0.51

[1] RI= Total gain using QTL – Total gain ignoring QTL

5.3.2 Selection on an index including production and disease susceptibility with equal emphasis on both traits

Under the additive model, polygenic and total gains for production were greater for schemes using the QTL (approximately 14% higher gains at generation 40 from using than from ignoring the QTL) when equal emphasis was placed on both traits in the index (Table 5.5). The opposite trend was seen for disease susceptibility where less gain was made in the schemes that used the QTL. Gains in the overall index were 5% higher when the QTL was used in selection. The frequency of the allele that reduced disease susceptibility was higher in the schemes that used the QTL (Figure 5.2a) and this led to a lower disease incidence in these schemes.

Similar results were obtained when the effect of the QTL on production was dominant (Table 5.6). At generation 40 the gain in the overall index was 6% higher in the schemes that used the QTL. As with the additive model, the frequency of allele B was higher in the scheme using the QTL (Figure 5.2b) and the disease incidence was lower until about generation 20.

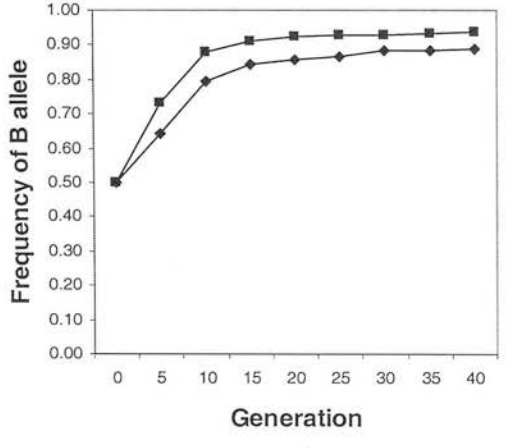
When the effect of the QTL on production was overdominant (Table 5.7), gains were higher for production (17% at generation 40) and lower for disease susceptibility when the QTL information was used. For the overall index 11% higher gain was achieved at generation 40 in the schemes that used the QTL. A higher B allele frequency was reached in these schemes, and the disease incidence in the population was lower until generation 40. The frequency of the allele approached equilibrium after about 14 generations for both schemes (Figure 5.2c).

Table 5.5. Polygenic (PG) and total (T) genetic means for production and disease susceptibility, index response (RI), and disease incidence (DI) across generations (Gen) using or ignoring the QTL when selection was on an index with equal emphasis on production and disease susceptibility (I_3) and the effect of the QTL on production was additive ($a_1 = 0.2$; $d_1 = 0$).

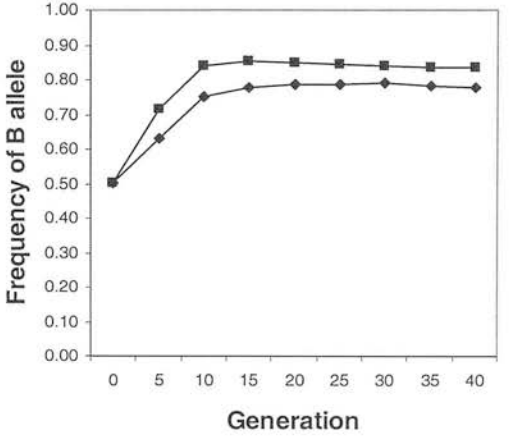
	Using QTL information					Ignoring QTL information					
	Production		Susceptibility			Production		Susceptibility			
Gen	PG	T	PG	T	DI	PG	T	PG	T	DI	RI[1]
0	0.00	0.00	0.00	-0.45	0.20	0.00	0.00	0.00	-0.45	0.20	0.00
1	0.25	0.17	-0.03	-0.77	0.10	0.26	0.21	-0.06	-0.68	0.16	0.05
2	0.54	0.43	-0.05	-0.86	0.07	0.50	0.43	-0.10	-0.81	0.12	0.05
3	0.85	0.72	-0.09	-0.94	0.06	0.75	0.67	-0.15	-0.90	0.11	0.09
4	1.17	1.03	-0.11	-0.96	0.06	1.01	0.92	-0.21	-0.98	0.09	0.09
5	1.47	1.33	-0.14	-1.00	0.05	1.27	1.16	-0.26	-1.05	0.08	0.12
10	2.09	1.93	-0.20	-1.07	0.04	1.81	1.69	-0.36	-1.17	0.06	0.14
20	5.06	4.89	-0.48	-1.36	0.02	4.43	4.28	-0.82	-1.67	0.02	0.30
30	7.90	7.73	-0.68	-1.57	0.01	6.92	6.76	-1.23	-2.09	0.01	0.45
40	10.56	10.38	-0.88	-1.77	0.01	9.26	9.10	-1.59	-2.45	0.00	0.60

[1] RI= Total gain using QTL – Total gain ignoring QTL

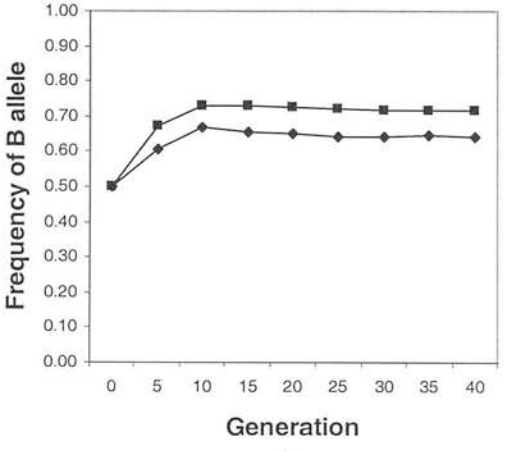
Figure 5.2. Frequency of the allele reducing susceptibility to disease (allele B) when using (■) or ignoring (♦) the QTL across generations when selection was on an index with equal emphasis on both traits and the mode of action of the QTL on production is additive (a), dominant (b) or overdominant (c).



a)



b



c)

Table 5.6. Polygenic (PG) and total (T) genetic means for production and disease susceptibility, index response (RI), and disease incidence (DI) across generations (Gen) using or ignoring the QTL when selection was on an index with equal emphasis on production and disease susceptibility (I_3) and the effect of the QTL on production was dominant ($a_1 = 0.2$; $d_1 = 0.2$).

	Using QTL information					Ignoring QTL information					
	Production		Susceptibility			Production		Susceptibility			
Gen	PG	T	PG	T	DI	PG	T	PG	T	DI	RI[1]
0	0.00	0.10	0.00	-0.45	0.20	0.00	0.10	0.00	-0.45	0.20	0.00
1	0.25	0.25	-0.03	-0.77	0.10	0.27	0.33	-0.05	-0.67	0.13	0.02
2	0.55	0.52	-0.06	-0.85	0.08	0.53	0.55	-0.10	-0.78	0.10	0.04
3	0.86	0.81	-0.09	-0.91	0.07	0.79	0.79	-0.14	-0.86	0.09	0.07
4	1.17	1.10	-0.12	-0.96	0.06	1.06	1.05	-0.20	-0.95	0.07	0.06
5	1.48	1.41	-0.15	-1.00	0.05	1.32	1.31	-0.24	-1.02	0.06	0.08
10	2.09	2.01	-0.20	-1.06	0.04	1.85	1.83	-0.33	-1.12	0.05	0.12
20	5.06	4.97	-0.49	-1.35	0.03	4.42	4.37	-0.77	-1.58	0.02	0.37
30	7.88	7.80	-0.71	-1.56	0.02	6.84	6.79	-1.18	-1.99	0.01	0.58
40	10.51	10.43	-0.90	-1.75	0.01	9.13	9.09	-1.54	-2.34	0.01	0.75

[1] RI= Total gain using QTL – Total gain ignoring QTL

Table 5.7. Polygenic (PG) and total (T) genetic means for production and disease susceptibility, index response (RI), and disease incidence (DI) across generations (Gen) using or ignoring the QTL when selection was on an index with equal emphasis on production and disease susceptibility (I_3) and the effect of the QTL on production was overdominant ($a_1 = 0.2$; $d_1 = 0.9$).

	Using QTL information					Ignoring QTL information					
	Production		Susceptibility			Production		Susceptibility			
Gen	PG	T	PG	T	DI	PG	T	PG	T	DI	RI[1]
0	0.00	0.45	0.00	-0.45	0.20	0.00	0.45	0.00	-0.45	0.20	0.00
1	0.25	0.55	-0.03	-0.77	0.10	0.23	0.62	-0.05	-0.65	0.14	0.05
2	0.57	0.87	-0.06	-0.80	0.09	0.46	0.84	-0.09	-0.75	0.14	0.08
3	0.86	1.14	-0.09	-0.86	0.08	0.70	1.06	-0.14	-0.81	0.12	0.13
4	1.17	1.44	-0.12	-0.90	0.08	0.94	1.28	-0.18	-0.86	0.11	0.20
5	1.47	1.73	-0.15	-0.92	0.07	1.17	1.50	-0.23	-0.91	0.10	0.24
10	2.05	2.31	-0.21	-0.98	0.07	1.65	1.98	-0.32	-1.02	0.10	0.29
20	4.90	5.16	-0.48	-1.24	0.05	3.98	4.32	-0.74	-1.41	0.06	0.67
30	7.61	7.89	-0.70	-1.45	0.04	6.20	6.56	-1.11	-1.77	0.04	1.01
40	10.19	10.47	-0.89	-1.65	0.03	8.26	8.62	-1.43	-2.09	0.02	1.41

[1] RI= Total gain using QTL – Total gain ignoring QTL

5.3.3 Selection against homozygotes for the allele increasing disease susceptibility

Table 5.8 shows the loss in genetic gain when animals that are homozygous for the allele that increases disease susceptibility and production (AA animals) are culled from the population before selection is applied on the index. Results for genetic gains and inbreeding are presented relative to those obtained when no culling of AA animals takes place.

Table 5.8. Percentage loss in total genetic mean in the index (% Loss I) and in production (% Loss P), frequency (Freq) of the allele increasing production (allele A), and the difference in inbreeding (F) in the population when AA animals were culled from the population, relative to when AA animals were included in selection when the mode of action of the QTL on production was additive, dominant or overdominant.

	I_1			I_3			
Gen	**% Loss I[1]**	**Freq**	**F**	**% Loss I**	**% Loss P**	**Freq**	**F**
			Additive (a_1=0.2;d_1=0.0)				
5	46	0.24	-0.03	36	37	0.24	-0.03
10	39	0.06	-0.06	21	25	0.06	-0.03
20	30	0.00	-0.06	18	18	0.02	-0.04
30	21	0.00	-0.06	9	11	0.00	-0.02
40	16	0.00	-0.06	6	7	0.00	-0.02
			Dominant (a_1=0.2;d_1=0.2)				
5	55	0.21	-0.02	43	55	0.21	-0.02
10	35	0.08	-0.04	33	39	0.08	-0.04
20	17	0.03	-0.03	24	32	0.01	-0.05
30	21	0.00	-0.05	14	22	0.00	-0.04
40	16	0.00	-0.04	8	15	0.00	-0.03
			Overdominant (a_1=0.2;d_1=0.9)				
5	37	0.30	-0.02	40	55	0.28	-0.02
10	27	0.11	-0.03	31	39	0.11	-0.03
20	37	0.01	-0.07	30	32	0.01	-0.07
30	22	0.00	-0.05	10	18	0.00	-0.07
40	13	0.00	-0.04	2	9	0.00	-0.05

[1] % Loss I = % Loss P for I_1.

Similar trends were observed for selection on the different indexes and modes of action of the QTL on production. As expected, regardless of the selection index, a substantial loss in the overall index and in production occurred when culling AA animals. A loss of 46%, 55%, and 37% occurred when the mode of action of the QTL on production was additive, dominant, and overdominant, respectively. When selection was on I_3 the loss in the index was less than the loss in production due to the improvement in disease resistance from culling susceptible animals. The culling strategy had the desired effect at eliminating the disease susceptibility allele in the population. The allele was eliminated more quickly when the QTL was additive. The mean inbreeding coefficient was 2% to 7% lower at every generation in the schemes where AA animals were culled.

5.4 Discussion

This study has evaluated the use of information on a pleiotropic biallelic QTL in selection when the mode of action of the QTL on the traits under selection differs. This has important implications in breeding programmes in light of the increasing number of pleiotropic QTL being detected in livestock species (see for example, Schrooten et al. 2004). We considered a QTL that had an effect on two economically important traits such as production and disease susceptibility. In all cases the QTL had a dominant mode of action on disease susceptibility, but the mode of action on production was either additive, dominant, or overdominant. The allele that increased production (i.e. A allele) also increased disease susceptibility. Artificial selection was applied on an index including production only or production and disease susceptibility, with either different or equal weights. In addition, animals that

reached a certain threshold for disease susceptibility were culled from the population. The value of using the QTL in breeding programmes in terms of genetic gain in the overall index depended on the mode of action of the QTL on production and the economic weights given to both traits in the index.

Most gene and marker assisted selection studies have considered QTL affecting only one trait, and have found an increase in response to selection when using the QTL in selection. However, as a result of various interactions at a molecular level it is likely that a single gene will have an influence on multiple traits (Lynch and Walsh, 1998). Few studies to date have looked at the benefits from including pleiotropic QTL in selection. De Koning and Weller (1994) looked at the efficiency of selection using the QTL relative to phenotypic selection (RSE) when a single QTL had an additive effect on two traits. When the genetic correlation between the two traits was zero and the QTL accounted for 30% of the genetic variance, the RSE was increased by 32% after one generation of selection. Here we found that when production was the only trait in the selection goal, the benefits of using the pleiotropic QTL depended on the mode of action of the QTL on this trait. When the QTL had an additive effect on production, genetic gain was similar for the first five generations but the scheme using the QTL achieved approximately 4% less gain by generation 40. In the study of De Koning and Weller, the QTL did not have a deleterious effect on either trait under selection, and increasing the frequency of the QTL did not affect the number of animals available for selection. In our study, animals that had high production were also more susceptible to disease and therefore selection of these animal led to a higher disease incidence and lower selection intensity when the QTL was used

compared to the situation where the QTL was ignored. A similar pattern was observed when the effect of the QTL on production was dominant. However, when the effect of the QTL on production was overdominant there was an increase in gain when the QTL was used in selection. In this situation there was little difference between the frequency either when the QTL was used or ignored (Figure 5.1c) and similar selection intensity was obtained in both schemes. The increased gain was attributable to the higher polygenic gain for production.

Over the last number of decades, selection in most livestock species has primarily focused on production related traits. However, such singular selection has resulted in many undesired correlated responses in other traits (Rauw et al., 1998). For instance, selection solely for milk yield in dairy cattle has resulted in major declines in cow fertility. Breeding programmes all over the world have acknowledged this decline and the breeding goals of many countries now include selection for non-production traits (Miglior, 2005) to arrest or reverse the decline in these traits. In this study we looked at the effect of using the QTL when both production and disease susceptibility are included in the selection index. In the first instance, there was five times more emphasis on production while in the second equal emphasis was given to both traits. When compared to selection on production only the most noticeable difference was that of disease incidence levels observed in the population. As expected, including disease susceptibility in the index led to a higher frequency of the allele reducing disease susceptibility (almost 0.9 for I_3) thereby reducing the disease incidence in the population when compared to selection on production only. For example, when the QTL had an additive effect on production and the QTL was

used, the disease incidence at generation 40 was 1% (Table 5.5) when disease was included in the index, compared to 10% (Table 5.2) when selection was on production only. When equal emphasis was placed on the traits the disease incidence in the population was halved after one generation of selection.

In all the selection scenarios considered the greatest additional response from using the QTL occurred when the QTL was overdominant, followed by the scenario where the QTL was dominant, while the least response was obtained when the mode of action was additive. When BLUP is used to estimate breeding values and genotype information is ignored, EBV will be biased as the EBV will be incorrectly regressed towards the parent average (Villanueva et al., 1999). This bias will be the greatest for an overdominant QTL, then a dominant QTL and lastly an additive QTL. Models leading to more bias will benefit more from the use of a BLUP that corrects for the effect of the QTL.

In this study we looked also at the effect of a more drastic strategy for decreasing disease susceptibility and that was to eliminate all animals homozygous for the disease susceptibility allele. As expected, the disease susceptibility allele was removed from the population but there was a substantial loss of gain (up to 55%) in the overall index. This was primarily due to the loss in production as a result of reduced selection intensity (approximately 25% less animals were available for selection in the initial generation). This reduced selection intensity is also likely to be the reason why the inbreeding in the schemes where all AA animals were culled was less than when they were not culled. Conversely, more gain was made in disease

susceptibility in schemes culling AA animals because more selection pressure was placed on this trait. The large reductions in gain for production may be regarded as an extremely high cost selection policy, and is probably not warranted in most situations, except were the cost of the disease is very high relative to production. Rather than using this drastic strategy for eliminating the allele conferring disease susceptibility, other approaches could be used to optimise selection using QTL information in multiple trait scenarios. Various techniques have been proposed for the optimisation of QTL in breeding programmes (e.g., Villanueva et al., 1999; Dekkers and Van Arendonk, 1999). These include optimising the contributions of selection candidates and optimising the weighting given to QTL across generations to maximise genetic gain at a given time horizon. Villanueva et al. (2004) looked at combining these two methods and found that substantial gains could be made by combining the two techniques. In the situation investigated in this study the task of optimisation is complicated by the genetic model assumed. On one hand there is a QTL which affects two traits in an antagonistic way. In addition, animals that have high production due to their QTL genotype are more likely to be culled because they are more susceptible to disease. One approach could be to use traditional selection index approaches. Here, the weightings of the traits in the selection indexes were chosen arbitrarily. In practice however, it should be possible to derive approximate economic values, based on the value of production and the costs of disease. These weights values could then be used in a selection index to achieve the required response.

5.5 Conclusions

In this chapter we have shown the potential outcomes from using a pleiotropic QTL in selection when the QTL has antagonistic effects on the two traits and one of the traits is directly related to the fitness of the animals. The results have shown that there can be different outcomes expected from using the QTL depending on its mode of action on both traits. The use of a pleiotropic QTL can, in some instances, lead to lower genetic gains than those achieved when ignoring the QTL information. It is clear that the use of QTL in any breeding scheme should be assessed carefully. In particular the effects and the nature of the QTL (i.e. affecting a single or multiple traits) should be ascertained as best as possible prior to selection. Once this has been determined the optimum use of the QTL in the breeding programme can then be examined.

CHAPTER SIX

General Discussion

Although quantitative genetics and animal breeding are long established areas, they are still evolving, maybe even more rapidly than ever. One subject of concern that has recently become the focus of attention is that of inbreeding. For example, intense selection of dairy bulls for milk production, combined with the ability to distribute semen from elite sires worldwide, has created a global population of Holsteins in which the rate of inbreeding is becoming of concern (Brotherstone and Goddard, 2005, Chapter 2), despite being the most numerically large breed. The ease at which semen can be distributed worldwide has meant that elite sires have thousands of descendants. The use of Best Linear Unbiased Prediction (BLUP) to estimate the genetic worth of animals has exacerbated the problem as BLUP tends to result in the co-selection of related animals. As a result, methods have been proposed to help control the problem of inbreeding while simultaneously increasing genetic gain (Meuwissen, 1997; Grundy et al., 1998).

The development of molecular genetic technologies has resulted in the sequencing of the complete genome of livestock species such as cattle and chicken, and has expanded our knowledge of the genes underlying genetic variation immensely. It is now possible to identify individual genes that are responsible for the variation in many traits. In the context of animal breeding, Quantitative Trait Loci (QTL) have been identified for many species and are been used in selection to complement existing breeding programmes.

In this thesis, the incorporation of new techniques in animal breeding programmes is assessed. Specifically we look at how the method that optimises genetic contributions

for maximising gain while restricting the rate of inbreeding could be used in the UK Holstein population. In chapter two, the inbreeding levels of the UK Holstein population (the largest dairy breed in the UK) are investigated. While the levels of inbreeding are not alarmingly high yet, we have shown that since the early nineties the rate of inbreeding has increased dramatically when compared to the preceding decade. The rate of inbreeding is a more informative measure of inbreeding than the level of inbreeding as the latter is dependent on the level of pedigree recording and the choice of the base population. It is interesting to note that the increase in the rate of inbreeding since the early nineties came rapidly after restrictions on the importation of semen were lifted. Several North American bulls had tens of thousands of daughters entering the recorded herd each year. These were bulls that were also very popular as "sires of sons" thus implying that the next crop of proven bulls were going to be very related to the current cow population. In addition, animal model BLUP was introduced as the method of genetic evaluation around this time also.

The consequences of inbreeding in dairy cattle have been outlined in several studies (e.g., Smith et al, 1998; Thompson et al., 2000; Wall et al., 2004). Currently the increase in inbreeding is approximately 1%/generation. This is similar to inbreeding rates seen in other countries such as the USA and Canada. Empirical evidence suggests that a rate of inbreeding per generation of 1% (which corresponds to an effective population size of 50) is an upper limit. Meuwissen and Woolliams (1994) showed that, in general, the fitness of a population decreases steadily when the effective population falls below 50. Indeed, this is the minimum population size that is suggested by the FAO (1998) for the development of a national conservation plan for endangered farm animal species. At the

moment the trend shows little signs of slowing down and is unlikely to do so until corrective measures are put in place at breeding programme level. One of the measures that could be taken is to apply optimised selection (Meuwissen et al., 1997; Grundy et al., 1998), a tool based on long-term contribution theory. In short, it has been shown that optimised selection is able to restrict the rate of inbreeding to predefined levels but, and maybe more importantly, can increase genetic gain at the same rate of inbreeding. Avendano et al. (2003) demonstrated its usefulness in the context of beef cattle and sheep breeding. Here we looked at the application of optimised selection at the level of the breeding companies as ideally this is where inbreeding should be tackled. In chapter two we compared the rate of inbreeding and genetic merit of the current crop of young bulls to a theoretical crop of young bulls that could have been generated from the same parents but using optimised selection. The results showed that the use of optimised selection could have achieved a higher genetic gain at the same rate of inbreeding, or conversely, could have achieved the same genetic gain but at ten times less inbreeding. Software has been developed in order to apply these techniques and several breeding companies are now using them (Brotherstone and Goddard, 2005). One of the difficulties of the application of these techniques is that they generally require the co-operation of breeding companies and breeders so that the rate of inbreeding can be controlled in the overall population. This is often not the case in the Holstein breed, where markets are controlled by several breeding companies. However, there is a good deal of consolidation among breeding companies throughout the world and in some countries the majority of the market is owned by a single company (e.g., LIC in New Zealand, Holland Genetic in the Netherlands), which should make the use of these techniques more attractive and manageable.

In the third chapter we developed a method to assess the economic benefit of using identified loci in commercial dairy herds. The large investment in QTL mapping means there will be an increasing number of genetic markers available, not only to breeding companies but also to commercial breeders, to aid selection. Currently, there are several such genetic markers available in dairy cattle such as DGAT1, bovine growth hormone, and leptin, with the likelihood of more being discovered. Before investing in such products it is important that their potential benefit is determined. Based on discounted gene-flow principles, a set of recursive equations were developed to quantify the value of using sires with a specific genotype for an identified gene in a commercial dairy herd. The inputs required are the probabilities of a cow surviving from one lactation to the next, the proportion of each genotype of the bulls used, the planning horizon, the discount rate, the initial frequency of the allele in the population, and the economic value of the allele. Two examples of identified loci were used to illustrate the usefulness of the method.

The first example dealt with the situation where bulls that are carriers of a genetic defect are selected to breed cows. This is of importance as often bulls that are carriers continue to be marketed after detection of the defect. We showed what the impact of using various proportions of carrier sires would be on a 100-cow herd and calculated a discount price that should be paid for semen of carrier sires. For example, a semen discount of £3.10 per CVM straw used would be required to offset the expected mortality when 20% of CVM carrier sires are used for 3 years when 5% of cows are carriers in the initial generation. The second example dealt with

increasing the frequency of a particular allele at a given locus that, when present in milk, increases the value of the milk. Such an allele is the A2 variant of β-casein as there is evidence to suggest that milk homozygous for this allele confers health benefits to consumers (Tailford et al., 2003). Interestingly, indigenous cattle breeds in Africa are almost all homozygous for the A2 allele (Aschaffenburg et al., 1968) and the prevalence of heart disease and juvenile diabetes are much lower in these countries. It is likely that the lower incidence of heart disease seen in African population can be attributed more to difference in the nutrition between third world and developed countries. The link to juvenile diabetes however cannot be as easily explained. Despite an unclear association between β-casein and health benefits (Swinburn, 2004), a premium of about 4-pence per litre is paid for A2A2 milk in New Zealand which could represent a significant increase in the value of milk over the lactation of a cow. This also represents one of the very few documented cases where producers actually receive premium for a product that can be selected for using genetic markers. Here we looked at the benefit of using A2A2 sires over a planning horizon of 20 years when A2A2 sires are used for one, five or 10 years. Even with a high discount rate of 20% (i.e. more value given to early expressions and less to later expressions as the long-term value may be reduced as more A2 milk is produced), the benefit of using an identified locus was clear. For example, assuming that an A2A2 cow is worth £160 more than a non-A2A2 cow over the course of one lactation, the expected benefit of using A2A2 sires for five years would be £57,120 for a 20 year planning horizon. The method is flexible in that it can accommodate different genetic models for the QTL, more than one QTL, and pleiotropic QTL.

Large quantities of resources are being employed to detect QTL. These QTL can help to understand the underlying biology of quantitative traits and can aid selection in breeding programmes. Indeed many livestock breeding companies are selecting on genetic markers or the QTL themselves. Many of these QTL have been detected using single trait analysis. However, it is important to understand the effect of the QTL may have on several important traits as this may impact upon the success of any MAS programme. Similar to the serious consequences experienced in Holsteins, whereby continual selection for production has led to a much undesired correlated decline in fertility and other fitness related traits, selection for QTL with a known effect on a particular trait but with unknown pleiotropic effects on other traits could have a negative impact on the population. In chapter four we looked at how deleterious alleles can be maintained in populations for many generations when selection has been solely on production. A practical scenario of this has been reported by Navarro et al. (2006) in a study of ascites in chickens. Despite an estimated zero additive genetic correlation between production and an indicator of ascites (blood oxygen saturation) they found a QTL that affected both traits. Also, they found that the disease-causing allele was at intermediate frequencies in the population, when it might be expected that natural selection would have removed the deleterious allele from the population. Their hypothesis was this could happen if selection was on production and there was a pleiotropic QTL that had a dominant effect on disease susceptibility and an overdominant effect on production. We showed that it was possible that a QTL affecting both production and disease susceptibility can remain segregating in the population for several hundred generations when the QTL allele favourable for production was unfavourable for

disease resistance and the mode of action of the QTL was dominant for disease resistance and either additive or dominant for production. When the QTL for production was overdominant the QTL reached equilibrium at an intermediate frequency. This would make it impossible to remove the deleterious allele by conventional selection. Therefore, it would be necessary to use markers to identify animals with the disease susceptibility allele in order to eliminate this allele from the population.

In chapter five the use of pleiotropic QTL in breeding programmes was studied. Few studies have looked at the use of pleiotropic QTL on genetic gain, and none have looked at the scenario where the QTL had a deleterious effect. Using similar models to those in chapter four, genetic gains and allele frequencies when using information on the QTL were compared to gains and frequencies from selection schemes that did not include information on the QTL. Most previous studies have reported higher gains, at least in the initial generations when using than when ignoring the QTL. In general, the genetic gain expected was dependent on the mode of action of the QTL on production. In this study, when selection was for production only and the QTL had an additive or dominant effect on production, less gain was made in this trait when the QTL was used. By using the QTL information in selection the frequency of the allele increasing disease susceptibility was increased and this resulted in a higher disease incidence in the population. This meant that the selection intensity and therefore the genetic gain for production were reduced. When the QTL had an overdominant effect on production greater genetic gain was achieved when the QTL was used than when it was ignored. In this situation the disease incidence in the

population was the same for both selection strategies. When disease susceptibility was also included in the selection index and equal emphasis was given to both traits, genetic gain in the index using the QTL were greater than when the QTL information was ignored. The effect of including disease susceptibility in the index meant that the disease resistance of the animals was improved more quickly and therefore the disease incidence in the population was similar when using and ignoring the QTL. These results have highlighted some important aspects of using QTL in breeding programmes, especially in terms of the effects of QTL on multiple traits and the mode of action of the QTL on the traits. The success of MAS will probably be due to several factors, not least the accurate detection and estimation of the size of QTL on several traits. In this thesis the polygenic correlation between production and disease susceptibility was assumed to be zero. If it were assumed that selecting for production did not have an impact on disease susceptibility as the estimate of the polygenic correlation would tend to suggest, selecting for that QTL and production alone could cause some unexpected results. More research at both the molecular and statistical level is being carried out to detect pleiotropic QTL. Ultimately this should lead to better predictions on the use of such QTL in breeding programmes.

Future Perspectives

Breeding companies need to continue to provide the highest quality genetic material to ensure their competitiveness in the market. Ultimately these benefits will be passed on to the breeder. In this respect inbreeding and the use of genetic markers are going to play a pivotal role over the next number of years. Past selection polices have resulted in more inbreeding in most livestock populations. Taking the example of

Holsteins, the decline in fertility has forced a move away from their use in several countries (e.g., Ireland, New Zealand) where fertility is vitally important due to seasonal calving patterns. Even in countries like the USA, Canada, and the Netherlands fertility is now an integral part of the national breeding goals. The decline could be due to the sole selection for increased yield, or inbreeding, or both. Holstein market share is being eroded by other breeds and crossbreeding in an effort to reverse the trends in fertility. To this end, a programme that produces bulls that do not produce highly inbred animals will be highly desirable from a breeder's perspective. The change in national breeding goals may help identify new bloodlines that were under-utilised in the past, however this is a sub-optimal strategy. The increased use of MAS could also lead to higher inbreeding. Just as the change in national selection goals may identify new bloodlines, similarly MAS may identify animals that are good for production and fertility and at an earlier stage in selection. This has the potential to multiply the existing inbreeding problems. The optimisation techniques discussed in chapter two have the potential to help manage these situations. They could form the basis of a very pro-active strategy that could be adopted by breeding companies. They have the ability to maximise genetic gain at a pre-defined rate of inbreeding which should prove worthwhile in both the short and longer term. The techniques are readily applicable to include markers in selection (Villanueva et al., 2004) and the inputs required are readily available from a national genetic evaluation (i.e. a pedigree file and estimated breeding values).

Much has been said and written about the role of molecular information in animal breeding programmes. Theoretically, MAS could yield up to 50 or 60% more gain

where the conditions are most favourable. However, despite this potential, the success of commercial application of MAS is unclear and not widely publicised. Dekkers (2004) states that while "initial expectations for the use of marker-assisted selection were high, the current attitude is one of cautious optimism". In general, most of the success of using genetic markers has been to test for single trait defects, and parentage identification. One of the drawbacks has been the time taken from the point of original detection to uncovering the functional mutation, which may be a slow and very costly process. The DGAT gene in dairy cattle is a prime example. It is likely that breeding companies will persist with the application of genetic markers in their selection programmes given the amount of resources that have already been devoted to identifying QTL. The mapping of the complete genomes and the rapid advances being made in molecular genetic technologies (e.g., gene expression arrays) to uncover the biological function and location of genes will not be ignored by breeding companies hoping to gain an advantage over their competitors. Much will depend on the economic aspects both in terms of how the marker/genes are detected and how they are applied thereafter. To be effective, the returns from using MAS will need to be greater than the costs. There are also implementation issues in the context of using markers in routine national genetic evaluations such as how to handle the markers, missing information and the need to modify existing software.

New techniques for high-throughput genotyping using single nucleotide polymorphisms (SNP) may provide a new approach to the application of marker assisted selection. The availability of these SNP at low cost could provide the path towards the implementation of genetic evaluations using molecular information

(termed "genomic selection") without actually identifying QTL or genes (Meuwissen et al., 2001). In essence, selection is based on a breeding value obtained as the sum of non-zero haplotype effects on particular traits. Accurate breeding values of animals can be obtained for animals that have no phenotypic record or their own and no progeny. This could lead to large increases in genetic gain. In order to successfully apply this, a dense marker map is required and low cost SNP genotyping may make this method more feasible. Genomic selection is currently been investigated by breeding companies but as yet there is no documentation on the success of this method in practice.

The uptake of new techniques has been shown to offer oppourtunites but also challenges. The framework and tools are in place if, for example, a breeding company wishes to use optimised selection techniques, not only to control the rate of inbreeding, but more importantly to maximise genetic gain. Depending on the structure of the breeding programme, the implementation issues may be minor or more difficult, nevertheless the benefits should be real. The integration of molecular genetic technologies along with traditional methods is likely to continue into the future, as the amount of information on individual genes and their effects are uncovered. We have shown how developing markers that can be marketed direct to commercial breeders can increase profitability of an enterprise. However, the widespread use of marker assisted selection should only be undertaken provided there is confidence in the identified markers, their effects, and their potential benefits.

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